



University
of Glasgow

<https://theses.gla.ac.uk/>

Theses Digitisation:

<https://www.gla.ac.uk/myglasgow/research/enlighten/theses/digitisation/>

This is a digitised version of the original print thesis.

Copyright and moral rights for this work are retained by the author

A copy can be downloaded for personal non-commercial research or study,
without prior permission or charge

This work cannot be reproduced or quoted extensively from without first
obtaining permission in writing from the author

The content must not be changed in any way or sold commercially in any
format or medium without the formal permission of the author

When referring to this work, full bibliographic details including the author,
title, awarding institution and date of the thesis must be given

Enlighten: Theses

<https://theses.gla.ac.uk/>
research-enlighten@glasgow.ac.uk

PROBLEMS ASSOCIATED WITH THE
ANALYSIS OF GLYPHOSATE
IN FOOD CROPS.

MOHTAR BIN YUSOF.

Thesis presented for
the degree of
Doctor of Philosophy
September, 1988.

Pesticide Chemistry,
Agricultural Chemistry Section,
Chemistry Department,
University of Glasgow.

ProQuest Number: 10998205

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10998205

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ACKNOWLEDGEMENTS.

I am much indebted to Dr. H.J. Duncan and Miss Isabella Boyd for their supervision of the work described in this thesis. Their advice and continual interest throughout the studies were greatly appreciated.

I wish to thank all the staff and my colleagues in the Agricultural Chemistry section, both past and present, who have been of valuable assistance in many ways, particularly in the field work. In addition, I am grateful to the Cell Biology Department for giving me permission and help to use the high speed centrifuge.

I am grateful to the Director General Department of Agriculture, Malaysia, Dato' Abu Bakar Mahmud and Public Service Department of Malaysia for study leave and financial support during the period of my study.

Finally, special thanks to my family especially my wife for their constant patience, support and encouragement throughout this work.

CONTENTS.

	Page no.
ACKNOWLEDGEMENTS	(i)
CONTENTS	(ii)
Summary	(vii)
A list of abbreviations used in this thesis	(xi)
CHAPTER 1 : INTRODUCTION	
1.1 Background	1
1.2 Reasons for investigation	11
1.3 Thesis objectives	18
CHAPTER 2 : ANALYSIS OF GLYPHOSATE RESIDUES IN FOOD CROPS ESPECIALLY POTATO	
2.1 Introduction	24
2.2 Detection of glyphosate by GC	26
2.2.1 Introduction	26
2.2.2 Experimental	28
2.2.3 Results and discussion	29
2.3 Detection of glyphosate by HPLC	34
2.3.1 Introduction	34
2.3.2 Experimental	36
2.3.3 Results and discussion	37
2.4 Extraction of glyphosate residue	39
2.5 Clean-up for glyphosate residue analysis	41
2.5.1 Survey of current clean-up methods	41

2.5.2	An assessment of various Bond-Elut ion exchange cartridges in retaining glyphosate from standards and samples	43
2.5.3	The use of various Bond-Elut cartridges and other materials in retaining co-extractives from samples	64
2.5.4	The use of activated carbon in retaining co-extractives from samples	69
2.5.4.1	Introduction	69
2.5.4.2	Material and method	70
2.5.4.3	Preliminary evaluation	71
2.5.4.4	Use of Norit Activated Carbon	82
2.5.4.5	Results and discussion	83
2.6	Recommended method for analysis of glyphosate residues in potatoes	91

CHAPTER 3 : EFFECT OF GLYPHOSATE ON FOOD TUBERS (POTATO).

3.1	Introduction	94
3.2	Field and storage experiment	98
3.2.1	Site details and plot layout	98
3.2.2	Application of glyphosate	98
3.2.3	Tuber storage	100
3.2.4	Residue analysis	101

3.2.5	Effect of glyphosate on tuber sprouting, number of eyes open and disease/ rotting	101
3.2.6	Subsequent field perfor- mance of glyphosate treated tubers	102
3.3	Results and discussion	103
3.3.1	Sprout length	105
3.3.2	Number of eyes open	112
3.3.3	Disease or rotting effect	118
3.3.4	Glyphosate residues in treated potato tubers	121
3.3.5	Subsequent field perfor- mance of glyphosate treated tubers	129
CHAPTER 4 : ANALYSIS OF GLYPHOSATE IN CEREAL GRAIN (BARLEY)		
4.1	Introduction	133
4.2	Modifications to the existing method of analysis	136
4.2.1	Detection	136
4.2.2	Extraction	137
4.2.3	Clean-up procedures	138
	4.2.3.1 Using Bond-Elut ion exchange cartridges	138

4.2.3.2	Using chelating and anion ex- change resins	139
4.3	Results and discussion	141
4.3.1	Using Bond-Elut ion exchange cartridges	141
4.3.2	Using chelating resin column	156
4.3.3	Charcoal as a clean-up material	167
4.3.4	Conclusions	170

CHAPTER 5 : DETERMINATION OF COMPLEX STABILITY CONSTANTS OF ALUMINIUM AND IRON (111) WITH GLYPHOSATE AND THEIR ENVIRONMENTAL IMPLICATIONS

5.1	Introduction	172
5.2	Experimental	177
5.2.1	Materials	177
5.2.2	Apparatus	179
5.2.3	Procedure	179
5.2.4	Calculations	180
5.3	Results and discussion	188
5.3.1	1:1 Aluminium chelate of glyphosate	195
5.3.2	Iron(111) chelate of glyphosate	197
5.4	Environmental implications	207

1. J. H. Van Vleck, *Phys. Rev.* **57**, 156 (1940).

2. J. H. Van Vleck, *Phys. Rev.* **58**, 116 (1941).

3. J. H. Van Vleck, *Phys. Rev.* **59**, 194 (1941).

4. J. H. Van Vleck, *Phys. Rev.* **60**, 152 (1942).

5. J. H. Van Vleck, *Phys. Rev.* **61**, 236 (1942).

6. J. H. Van Vleck, *Phys. Rev.* **62**, 305 (1942).

7. J. H. Van Vleck, *Phys. Rev.* **63**, 379 (1943).

8. J. H. Van Vleck, *Phys. Rev.* **64**, 458 (1943).

9. J. H. Van Vleck, *Phys. Rev.* **65**, 541 (1944).

10. J. H. Van Vleck, *Phys. Rev.* **66**, 624 (1944).

11. J. H. Van Vleck, *Phys. Rev.* **67**, 712 (1945).

12. J. H. Van Vleck, *Phys. Rev.* **68**, 800 (1945).

13. J. H. Van Vleck, *Phys. Rev.* **69**, 889 (1946).

14. J. H. Van Vleck, *Phys. Rev.* **70**, 978 (1946).

15. J. H. Van Vleck, *Phys. Rev.* **71**, 1067 (1947).

16. J. H. Van Vleck, *Phys. Rev.* **72**, 1156 (1947).

17. J. H. Van Vleck, *Phys. Rev.* **73**, 1245 (1948).

18. J. H. Van Vleck, *Phys. Rev.* **74**, 1334 (1948).

19. J. H. Van Vleck, *Phys. Rev.* **75**, 1423 (1949).

20. J. H. Van Vleck, *Phys. Rev.* **76**, 1512 (1949).

21. J. H. Van Vleck, *Phys. Rev.* **77**, 1601 (1950).

22. J. H. Van Vleck, *Phys. Rev.* **78**, 1690 (1950).

23. J. H. Van Vleck, *Phys. Rev.* **79**, 1779 (1951).

24. J. H. Van Vleck, *Phys. Rev.* **80**, 1868 (1951).

25. J. H. Van Vleck, *Phys. Rev.* **81**, 1957 (1951).

26. J. H. Van Vleck, *Phys. Rev.* **82**, 2046 (1951).

27. J. H. Van Vleck, *Phys. Rev.* **83**, 2135 (1952).

28. J. H. Van Vleck, *Phys. Rev.* **84**, 2224 (1952).

29. J. H. Van Vleck, *Phys. Rev.* **85**, 2313 (1952).

30. J. H. Van Vleck, *Phys. Rev.* **86**, 2402 (1952).

31. J. H. Van Vleck, *Phys. Rev.* **87**, 2491 (1952).

32. J. H. Van Vleck, *Phys. Rev.* **88**, 2580 (1952).

33. J. H. Van Vleck, *Phys. Rev.* **89**, 2669 (1952).

34. J. H. Van Vleck, *Phys. Rev.* **90**, 2758 (1952).

35. J. H. Van Vleck, *Phys. Rev.* **91**, 2847 (1952).

36. J. H. Van Vleck, *Phys. Rev.* **92**, 2936 (1952).

37. J. H. Van Vleck, *Phys. Rev.* **93**, 3025 (1952).

38. J. H. Van Vleck, *Phys. Rev.* **94**, 3114 (1952).

39. J. H. Van Vleck, *Phys. Rev.* **95**, 3203 (1952).

40. J. H. Van Vleck, *Phys. Rev.* **96**, 3292 (1952).

41. J. H. Van Vleck, *Phys. Rev.* **97**, 3381 (1952).

42. J. H. Van Vleck, *Phys. Rev.* **98**, 3470 (1952).

43. J. H. Van Vleck, *Phys. Rev.* **99**, 3559 (1952).

44. J. H. Van Vleck, *Phys. Rev.* **100**, 3648 (1952).

45. J. H. Van Vleck, *Phys. Rev.* **101**, 3737 (1952).

46. J. H. Van Vleck, *Phys. Rev.* **102**, 3826 (1952).

47. J. H. Van Vleck, *Phys. Rev.* **103**, 3915 (1952).

48. J. H. Van Vleck, *Phys. Rev.* **104**, 4004 (1952).

49. J. H. Van Vleck, *Phys. Rev.* **105**, 4093 (1952).

50. J. H. Van Vleck, *Phys. Rev.* **106**, 4182 (1952).

51. J. H. Van Vleck, *Phys. Rev.* **107**, 4271 (1952).

52. J. H. Van Vleck, *Phys. Rev.* **108**, 4360 (1952).

53. J. H. Van Vleck, *Phys. Rev.* **109**, 4449 (1952).

54. J. H. Van Vleck, *Phys. Rev.* **110**, 4538 (1952).

55. J. H. Van Vleck, *Phys. Rev.* **111**, 4627 (1952).

56. J. H. Van Vleck, *Phys. Rev.* **112**, 4716 (1952).

57. J. H. Van Vleck, *Phys. Rev.* **113**, 4805 (1952).

58. J. H. Van Vleck, *Phys. Rev.* **114**, 4894 (1952).

59. J. H. Van Vleck, *Phys. Rev.* **115**, 4983 (1952).

60. J. H. Van Vleck, *Phys. Rev.* **116**, 5072 (1952).

61. J. H. Van Vleck, *Phys. Rev.* **117**, 5161 (1952).

62. J. H. Van Vleck, *Phys. Rev.* **118**, 5250 (1952).

63. J. H. Van Vleck, *Phys. Rev.* **119**, 5339 (1952).

64. J. H. Van Vleck, *Phys. Rev.* **120**, 5428 (1952).

65. J. H. Van Vleck, *Phys. Rev.* **121**, 5517 (1952).

66. J. H. Van Vleck, *Phys. Rev.* **122**, 5606 (1952).

67. J. H. Van Vleck, *Phys. Rev.* **123**, 5695 (1952).

68. J. H. Van Vleck, *Phys. Rev.* **124**, 5784 (1952).

69. J. H. Van Vleck, *Phys. Rev.* **125**, 5873 (1952).

70. J. H. Van Vleck, *Phys. Rev.* **126**, 5962 (1952).

71. J. H. Van Vleck, *Phys. Rev.* **127**, 6051 (1952).

72. J. H. Van Vleck, *Phys. Rev.* **128**, 6140 (1952).

73. J. H. Van Vleck, *Phys. Rev.* **129**, 6229 (1952).

74. J. H. Van Vleck, *Phys. Rev.* **130**, 6318 (1952).

75. J. H. Van Vleck, *Phys. Rev.* **131**, 6407 (1952).

76. J. H. Van Vleck, *Phys. Rev.* **132**, 6496 (1952).

77. J. H. Van Vleck, *Phys. Rev.* **133**, 6585 (1952).

78. J. H. Van Vleck, *Phys. Rev.* **134**, 6674 (1952).

79. J. H. Van Vleck, *Phys. Rev.* **135**, 6763 (1952).

80. J. H. Van Vleck, *Phys. Rev.* **136**, 6852 (1952).

81. J. H. Van Vleck, *Phys. Rev.* **137**, 6941 (1952).

82. J. H. Van Vleck, *Phys. Rev.* **138**, 7030 (1952).

83. J. H. Van Vleck, *Phys. Rev.* **139**, 7119 (1952).

84. J. H. Van Vleck, *Phys. Rev.* **140**, 7208 (1952).

85. J. H. Van Vleck, *Phys. Rev.* **141**, 7297 (1952).

86. J. H. Van Vleck, *Phys. Rev.* **142**, 7386 (1952).

87. J. H. Van Vleck, *Phys. Rev.* **143**, 7475 (1952).

88. J. H. Van Vleck, *Phys. Rev.* **144**, 7564 (1952).

89. J. H. Van Vleck, *Phys. Rev.* **145**, 7653 (1952).

90. J. H. Van Vleck, *Phys. Rev.* **146**, 7742 (1952).

91. J. H. Van Vleck, *Phys. Rev.* **147**, 7831 (1952).

92. J. H. Van Vleck, *Phys. Rev.* **148**, 7920 (1952).

93. J. H. Van Vleck, *Phys. Rev.* **149**, 8009 (1952).

94. J. H. Van Vleck, *Phys. Rev.* **150**, 8098 (1952).

95. J. H. Van Vleck, *Phys. Rev.* **151**, 8187 (1952).

96. J. H. Van Vleck, *Phys. Rev.* **152**, 8276 (1952).

97. J. H. Van Vleck, *Phys. Rev.* **153**, 8365 (1952).

98. J. H. Van Vleck, *Phys. Rev.* **154**, 8454 (1952).

99. J. H. Van Vleck, *Phys. Rev.* **155**, 8543 (1952).

100. J. H. Van Vleck, *Phys. Rev.* **156**, 8632 (1952).

SUMMARY.

The principle of this thesis concentrates particularly on developing a simpler method of determination of glyphosate at residue level with the emphasis on clean-up procedures. The difficulties of obtaining a simple method of quantitation of this compound are mainly due to its properties; its relatively high solubility in water, its insolubility in organic solvents and its complexing behaviour. These characteristics are quite similar to compounds naturally existing in plants or crops and thus make it difficult to isolate from these matrices. Its properties were investigated in detail in order to predict its behaviour more accurately. The work can be sub-divided as follows :

1. A brief discussion was made of the chemistry of glyphosate and the difficulties involved when it is quantified by GC or HPLC mainly because of the derivatization problem involved. The current methods of determination of glyphosate residues in various matrices were also presented as a reference. The justification of the choice of food crops as a key study were mentioned.
2. Analysis of glyphosate residues in a tuber food crop (potato).

The main aim of this work was to develop a simple clean-up procedure for the determination of glyphosate residues in food crop (potato) that could be used for detection both by GC and HPLC. It was found that

determination by GC especially with an EC detector involved simultaneous esterification and acylation and was not suitable for this purpose mainly because the derivative retention time was relatively short and easily masked by the impurities. Due to the glyphosate solubility behaviour, HPLC was advantageous over GC and was chosen for all subsequent detection.

Simple clean-up procedures were developed for the determination by HPLC and pre-column derivatization was preferred because it was less complicated than determination by post-column derivatization.

All types of Bond-Elut small cartridges ranging from non-polar to anion exchange were not effective in retaining glyphosate from potato extracts, although anion exchange cartridges could strongly retain glyphosate standards.

A mixture of semi coarse and powdered forms of activated carbon was found to be useful as a clean-up material to adsorb impurities from the potato extract. Recovery was found to be 53 % at 0.1 ppm sample and up to 92 % at 10 ppm sample with the limit of detection at 0.05 ppm. This could be considered a reasonably good recovery and a good alternative procedure to those currently employed.

3. The translocated effect of glyphosate to the tuber crops was simulated by spraying it onto the plant leaves of a tuber crop (potato). The physical effects on the tubers were observed under normal storage conditions. Results showed that sprout lengths were reduced, number of

eyes open and rotting increased with increasing rate of glyphosate application. The residue content in the tubers also increased with increasing amount of glyphosate applied.

For the assessment of the injuries caused by glyphosate, all the above parameters could be taken into consideration. However better accuracy could be obtained if the rotting effect and glyphosate residue were used as references. If the rotting effect was to be used for the assessment all the possible diseases should be determined first in order to avoid the difficulty of differentiating their symptoms.

Field growth performance indicated that all the treated tubers were affected by glyphosate even at the lowest treatment level (0.09 kg/ha glyphosate). Tubers from the lowest treatment level did not show any significant difference compared with controls in terms of sprout length, number of eyes open, rotting and residue level during storage. From this work, residue levels of 0.1 ppm or above had a decided effect on subsequent growth. Treatment with ≥ 0.36 kg/ha glyphosate which gave significant effects during storage did not produce any healthy plants or daughter tubers when planted out.

4. A small comprehensive exercise on the determination of glyphosate in barley grain was undertaken. Adaptations of the experiments in chapter 2 were applied. It was found that anion exchange Bond-Elut cartridges could retain glyphosate in barley extracts better than in potato extracts. However its recovery was still relatively low

to enable its use as a clean-up material.

A chelating resin was also tried to retain glyphosate from barley extract. Its recovery was 52 % at 1 ppm and 42 % at 0.1 ppm level. Formation of gel during the extraction could reduce recovery and attempts on how to eliminate it were also mentioned.

5. Complexing properties of glyphosate that had been utilised in the residue analysis in chapter 4 were studied in further detail. Attempts to determine complex stability constants for glyphosate with aluminium and iron(III) were tried in perchlorate (inert) medium. It was found that glyphosate formed a stable complex with aluminium and its log stability constant was 13.04. Glyphosate and iron(III) formed a precipitate in this medium. However, glyphosate and iron(III) did not form any precipitate in a nitrate medium. Preliminary experiments were performed to suggest the composition of this precipitated compound.

The environmental implications of the above experiments including glyphosate chelating properties were investigated especially in relation to the deactivation of glyphosate in the environment. Thus the fate of glyphosate in the environment could be further explained and predicted especially that which related to its herbicide residual activity.

6. Some conclusions as to the outcome of the studies were drawn. The implications of the above studies were discussed and some suggestions for further investigation were also highlighted.

A list of abbreviations used in this thesis.

AAS	atomic absorption spectrometry
ca.	circa (about)
cm	centimetre
cv.	cultivar
df	degrees of freedom
ed(s).	editor(s)
et al.	and others (authors)
g	gram
GC	gas chromatography
ha	hectare
HPLC	high performance liquid chromatography
i.e.	that is
kg	kilogram
l	litre
LSD	least significant difference
m	metre
M	molar
meq	milliequivalent
mg	milligram
min.	minute
ml	millilitre
ng	nanogram (10^{-9} gram)
ppm	part per million
ug	microgram (10^{-6} gram)
ul	microlitre
no.	number
ns	not significant

n.a.	data not available
p	statistical probability
sec.	second
s.d.	standard deviation
viz.	namely
%	percentage
°C	degree centigrade
>	greater than
≥	greater than or equal to
>>	much greater
<	less than
≤	less than or equal to
~	about

CHAPTER 1.

INTRODUCTION.

1.1 BACKGROUND.

In world agrochemical terms, herbicides have the highest sale value where its stake was around £ 7200 million or 40 % of the world sale in 1984. Other agrochemicals such as insecticides, fungicides and others were around £ 5940, 3780 and 1080 million respectively (ICI Plant Protection Division,U.K.).

The herbicide glyphosate sold commercially as Round-up, is a post-emergence non selective herbicide, introduced in 1971. It is being sold to more than 119 countries and is labelled for use on more than 50 agricultural crops and industrial sites (Grossbard and Atkinson,1985). It is a translocated herbicide along with other herbicides introduced earlier such as 2,4-D, aminotriazole, asulam and dalapon which are also in this group (Hassall,1982).

Glyphosate is a popular choice because it exhibits many unique biological properties including the following :

- i . There is no other commercial herbicide available which exhibits such diverse utility and efficacious performance in the control of almost all annual, biennial and perennial grassy and broad leaf weeds.

It effectively controls 76 of the world's 78 worst weeds (Franz, 1985).

- ii. It does not sterilize treated soil and is readily metabolised in the soil by microorganisms to natural products (Sprankle et al., 1975; Rueppel et al., 1977).
- iii. It is relatively non-toxic to other life forms.
LD₅₀ = 4300 mg/kg for rat (EPA, 1982a).
- iv. There is relative low risk of environmental pollution (Miles et al., 1986).

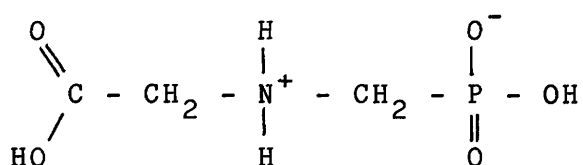
The properties that make this compound effective include its relatively high water solubility, rapid foliar absorption and translocation by plants and a low degree of in vivo metabolism and degradation (Guinivan et al., 1982).

The unique herbicidal characteristics of glyphosate are surprising and could not have been predicted especially at the beginning of its discovery. It has high unit activity. Since the discovery of glyphosate's unique herbicidal activity, hundreds of derivatives, homologues, analogues and related compounds have been prepared and evaluated in suitable screens. All analogues, homologues and isoesters of glyphosate lack similar types of activity. The results generally indicate that glyphosate acid and its water soluble salts were the compounds of highest unit activity (Franz, 1974).

Due to the limited solubility of its acid in water, 1.2 % at 25°C (Anon, 1971), soluble derivatives were preferred for formulation purposes because the free acid

is inconvenient to use. For commercial formulation, the monoisopropylamine salt with a wetter of undisclosed type were used. It is commonly recognised that glyphosate and its ionic forms are biologically equivalent.

Glyphosate or its chemical name N - (phosphonomethyl) glycine is a white crystalline solid. In the solid state it exists as a zwitterionic species (Knuuttila and Knuuttila,1979).



Glyphosate in solid state

In aqueous solution one hydrogen atom alternates between the phosphono and amino groups at such high frequency that its location cannot be determined.

Glyphosate acid has low solubility in water, 1.2 to 8 % at 25 - 100 °C and is insoluble in organic solvents. This is due to its strong inter molecular hydrogen bonding in the crystalline lattice (Knuuttila and Knuuttila,1985). It was found that pure glyphosate was stable for many years in distilled water or in 1 M HCl at room temperature. Spray solutions of formulation product Round-up, stored in glass bottles at room temperature, did not change noticeably in 7 months. The tendency to hydrolytic decomposition was also low or non existent (Friedstad,1978).

It has an acidic property in water where the pH of a 0.0067 M solution of glyphosate in 0.05 M KCl is 2.5. Complete conversion of glyphosate to monoanion, dianion and trianion occurs at pH values approximately 4, 8 and 12 respectively (Wauchope, 1976). These indicated that at least the alkali metals can form mono, di and tri - salts. Although glyphosate is acidic in water, it also has an amphoteric nature where it will dissolve in strong acids to produce salts with negative pK_a values and crystalline hemisalts have been isolated (Franz, 1974).

Glyphosate possesses distinctive properties compared to most other pesticides especially when it is dealt with at low residue levels. This compound is closely related to the exceptionally polar ones in which its characteristic is relatively high solubility in water but insoluble in organic solvents.

There has been significant interest in recent years in analytical residue methodology for glyphosate as a result of the increased usage of this herbicide (Cowell et al., 1986). For quantitation of this compound, problems arise because of its solubility properties. It needs to be extracted with water and in an aqueous mixture the compound is normally difficult to clean-up and converted to a volatile molecule for Gas Chromatography analysis (Guinivan et al., 1982).

Three functional groups of glyphosate, phosphonic acid, carboxylic acid and secondary amine also give problems for derivatization, especially to produce more volatile compounds for the purpose of gas chromatography

analysis. Glyphosate could not be analysed especially at residue level without any derivatization due to the above mentioned properties and also because it is ultraviolet inactive even at 200 nm and it lacks a conjugated system for fluorescence detection (Bardalaye et al.,1985).

Various approaches to the analysis of glyphosate at residue level mainly rely on derivatizing partly or all its functional groups. Most of the work has been hampered by the fact that glyphosate is only soluble in water. This characteristic makes conventional acylation and esterification reactions which are typically performed in anhydride - acid mixtures and ethyl ether respectively (Blau and King,1978) slow or in some cases impossible to perform. The derivatization is further slowed down for the residue sample because some substrate co-extractives may form a film at the bottom of the reaction vessel restricting reagent - reactant contact. Some attempts have been made to fully solubilize glyphosate for derivatization using conventional solvents but this has been unsuccessful (Bardalaye et al.,1985).

The currently known methods of analysis for glyphosate especially at residue level, namely gas-liquid chromatography (GLC) with flame photometric detector (FPD) or electron capture detector (ECD), gas chromatography/mass spectrometry (GC/MS) with flame ionization detector (FID), high performance liquid chromatography (HPLC), thin layer chromatography (TLC) etc. are summarized in table 1.2.

Table 1.2 Methods for analysis of glyphosate.

Method	Extractant	Clean-up	Derivatization	Detection	Application, level, (recovery).	Reference
GC	1.Pre-extraction with organic solvents.	1.Strong cation exchange resin.	Methyl N-trifluoro acetyl ester.	FPD	Almost all known food crops, animal tissues, soil,water,etc., 0.1 ppm (45-60%).	Monsanto (PAM 1977)
	2.Water.	2.Chacoal.				
		3.Strong anion exchange resin.				
GC	Water.	1.Liquid-liquid partitioning.	2-chloroethyl-N-heptafluorobutyryl ester.	ECD	Blue berries, 0.01 ppm, (43-61%).	Guinivan, Thompson and Wheeler (1982).
		2.Gel Permeation chromatography.				
		3.Cation exchange column.				
GC/HPLC	Water Chloroform Mixture.	1.Anion exchange resin.	Methyl-N-trifluoro acetyl ester.	FPD	Kiwi fruit and asparagus, 0.1 ppm (80.- 87%).	Seiber,McChesney, Kon and Leavit (1984).
		2.Gel Permeation Chromatography.				
		3.HPLC for derivatized compound.				

GC/MS	-	-	n-butyl-N-tri-fluoro acetyl ester.	FID	Standard only	Rueppel, Suba and Marvel (1976). Moye and Deyrup (1984).
GC/MS	-	-	Dimethyl tert-butyl-silyl ester.	FPD	Standard only	
HPLC	-	-	No derivatization, intact compound.	Ultraviolet, refractive index.	Glyphosate in formulation and technical samples, detection limit: 2.5µg/injection	Burns and Tomkins (1979).
HPLC-post column derivatization.	Water-Chloroform Mixture.	cation exchange resin.	Derivatization for fluorogenic labelling.	Fluorescence	Cantaloupe, cranberries, cucumbers, pumpkins, blue berries, squashes, 0.1 ppm (70-96).	Moye and St. John (1980). Moye, Miles and Scherer (1983).
HPLC-post column derivatization.	0.1 M HCl-chloroform Mixture	1.Chelex resin Fe(III) form. 2.Anion exchange resin.	Derivatization for fluorogenic labelling.	Fluorescence	Soya bean, grape, cabbage, alfalfa, water, 0.05-5 ppm (21.4-135.5).	Cowell et al., (1986)
HPLC-post column derivatization.	Water.	AX/Corasil Bondapack pre-column.	Derivatization for fluorogenic labelling.	Fluorescence	Barley, wheat, rye, vegetable, 1 ppm (80%).	Tuinstra and Kienhuis (1987).

HPLC- post column deriva- tization.	Water.	Ion exchange chromato - graphy.	Derivatization for fluorogenic labelling.	Fluorescence	Apple,grape, tea leaves, soya bean plants, detection limit:0.5 ng (reproducibi- lity 62 - 64%).	Nazarova, Dolinskaya, Makeev, Chkanikov (1988).
HPLC pre- column deriva- tization.	Water- Chloroform Mixture.	Cation ex- change resin.	Derivatization for fluoro- nic labelling.	Fluorescence	Straw, 0.1 ppm (70 - 80 %).	Roseboom Berkhoff (1982).
HPLC pre- column deriva- tization.	Water.	Anion ex- change resin.	Derivatization for fluoro- nic labelling.	Fluorescence	Soil,water, 25 - 200 ppm (20 - 55 %).	Glass (1983).
HPLC pre- column deriva- tization.	-	-	Derivatization for ester fluo- rogenic label- ling.	Ultraviolet	Standard, ~1 µg/in- jection.	Huber 111 and Calabrese (1985).
HPLC pre- column deriva- tization.	0.1 M tri- ethyl amine.	Anion ex- change resin.	Derivative of N-2,4 dinitro benzene.	Ultraviolet	Soil, 1.43 ppm (56 - 93%).	Lundgren (1986).
HPLC pre- column de- rivatiza- tion.	-	-	Derivatization for fluoro- nic labelling.	Fluorescence	Water, 0.05 ppm (76 %).	Miles, Wallace and Moye (1986)

HPLC pre- column deriva- tization.	0.1 M KH_2PO_4 or 0.2 M KOH.	-	Derivatization for fluoro- genic labelling.	Fluorescence	Soil, 0.5 ppm (35 - 100%).	Miles and Moye (1988).
HPLC	Adsorption studies.	-	No derivati- zation.	Ultraviolet.	Clay - glyphosate standard, -	Mc Connell and Hossner (1985).
TLC	Water.	Florisil	No derivati- zation.	Fluorescamine reagent.	Cirsium arvense root, 5 ppm (75%).	Young, and Marriage (1977).
TLC	Water.	-	No derivati- zation.	Spraying with amine speci- fic reagent.	Water and soil, detection limit: 0.05 ppm.	Pavoni (1978).
TLC	Water.	-	No derivati- zation.	0.5 % ninhy- drin in butanol.	Bindweeds, -	Sprankle et al., (1978).
TLC	-	-	No derivati- zation.	Ninhydrin, copper nitrate and Rhodamine B.	Water detection limit: 100 ng.	Ragab (1978).
TLC	-	-	No derivati- zation.	Ninhydrin.	Water, sensitivity 2 µg/spot.	Gitsova Bratano (1984).
TLC	Water.	Selective extraction and clean- up.	No derivati- zation.	Ninhydrin.	Soil, corn, grape, water, air, detec- tion limit : 0.01 ppm.	Bunyatyan and Gevorgyan (1984).

TLC	Water.	-	No derivatization.	Ninhydrin	Biological materials, 0.4 µg/zone. Water, 1 ppm. (94 - 99%).	Topalski and Vulera (1985). Glass (1981).
Colourimetric.	-	-	Phosphomolybdate heteropoly blue complex.	Colourimetric.		
Amino acid analyzer.	-	Cation exchange resin.	Derivatization with ninhydrin.	Colourimetric.	Standard detection limit : 3.4 µg.	Ekstrom & Johansson (1975).
Amino acid analyzer.	Water.	DS - 6A column.	Derivatization with ninhydrin.	Colourimetric.	Apple, grape, tea leaves, soya bean plants detection limit: 0.06 µg (reproducibility 62-64%).	Nazarova, Dolinskaya, Makeev, Chkanikov and Pavlova (1988).
Polarographic.	-	Anion exchange resin.	Derivatization to nitroso compound.	Polarographic.	Water detection limit : 0.35 ppm.	Bronstad and Friestad (1976).
Polarographic.	Water (crops) dilute KOH (soil).	1. Anion exchange resin 2. Charcoal.	Derivatization to nitroso compound.	Polarographic.	Blue berries, cowberries, raspberries, barley, oats, wheat, potato, soil, water, 2 ppm (63-86%) 0.05 ppm (76%) for water. Distilled water, detection limit: 500 ppm.	Friestad and Bronstad (1982). Ragab, Stiles and Yeo (1976)
Molecular emission cavity.	-	-	-	-		

1.2 REASONS FOR INVESTIGATION.

As mentioned earlier herbicides have the highest of the pesticide world sale value (1984). The sale value of herbicides also increases year by year (in the U.K) as indicated in table 1.1. No specific information could be obtained on herbicide glyphosate (Round-up) production (Monsanto,U.K), but it has been labelled for use on more than 50 agricultural crops and industrial sites and it is marketed in more than 119 countries. It could be predicted that the use of this herbicide will increase year by year. Cowell et al., (1986) indicated that the use of Round-up, Bronco and Rodeo (commercial formulation) were increasing.

Table 1.1 Sales of pesticide in the U.K. (£m) in
1977 - 81 (from Elliot and Wilson,1983)

Product type	1977	1978	1979	1980	1981
Herbicide	72.9	98.9	134.1	125.8	143.1
Insecticide	21.8	20.5	23.0	22.0	23.9
Fungicide	16.0	20.4	34.2	38.7	52.0
Seed treatment products	2.1	5.4	5.8	5.7	6.8
Growth regulator	n.a.	n.a.	n.a.	3.4	2.5
Herbicide/fertiliser mixtures	1.9	2.5	3.7	2.7	2.9
Ectoparasitcides	3.1	4.0	5.4	2.8	2.8
Other pesticides	3.8	4.6	6.0	5.3	8.0

n.a = not available

The increasing use of this herbicide also could be judged from the fact that glyphosate as a relatively new chemical was introduced at a price the market would bear for the priority uses and markets on which the discovering company initially concentrated its attention. This practice was aimed at maximising the chance of recovering the high cost of research and development within a reasonable period of time. Glyphosate is now assuredly through this phase and into one where it can be available at a price for an effective dose which permits its consideration for a very wide range of uses throughout the world, both in countries with highly mechanised cost effective agricultural production technologies and in developing countries with a variety of other simpler agricultural practices (Holly,1985).

Increasing use of this chemical, has created significant interest in recent years in its analytical residue methodology. Many investigators have found that the Pesticide Analytical Manual (PAM) methods (FDA,1980) for measuring glyphosate residues on crops and other vegetation were inadequate. Consequently new methods have been developed (Moye and St.John,1980; Guinivan et al.,1982; Moye et al.,1983; Glass,1983; Bardalaye et al.,1985; Lundgren, 1986; Cowell et al.,1986; Miles and Moye,1988). Generally it is accepted that the analysis of glyphosate residues is far from facile (Bardalaye et al., 1985).

From table 1.2 it can be seen that there are many methods of detection of glyphosate in various matrices. However the investigation concentrated on using gas chromatography (GC) and high performance liquid chromatography (HPLC) because other methods of detection such as thin layer chromatography, polarographic and colorimetric are less sensitive or semi quantitative. Another reason was that these two instruments are widely used in pesticide residue analysis.

Another characteristic of glyphosate is that it is relatively soluble in water as mentioned earlier but insoluble in organic solvents. Its partition coefficient between n-octanol and water is 6×10^{-4} at a concentration of 20 mg glyphosate/litre (Tooby,1985). For residue analysis it must be extracted from matrices with water for the above reasons. When water, a polar solvent, is used as extractant, it will extract more compounds or impurities compared to organic solvents. Water will extract a wide range of compounds, from less polar to ionic, whereas organic solvents will only extract compounds which are quite similar to their polarity index (Synder and Kirkland,1979). Hence it can be predicted that water extracted compounds will require intensive clean-up procedures prior to detection. The pure chemical or a clean sample with relatively low impurities causes few problems in analysis.

Since glyphosate extracts will contain many impurities and its subsequent clean-up could not be done with organic solvents because of the nature of the

impurities extracted, the difficulty of the analysis will essentially lie in the clean-up (Bardalaye et al.,1985). It seems that the only method normally requiring no clean-up procedure was TLC but this method was semi quantitative (Moye et al.,1983). In the latest development on analysis by TLC which was more sensitive than the earlier ones, selective extraction and clean-up were required as many compounds gave colour reactions with ninhydrin (Bunyatyan and Gevorgyan,1984).

Little in the way of information is available on the amount of glyphosate used on crops in the U.K. Hence the break down of herbicide usage on major crops in the U.K. in 1981 can be seen in table 1.3.

Table 1.3 Herbicide usage on major crops in the U.K. in 1981 (thousand hectares) (Elliot and Wilson,1983).

Type of crop	area	Herbicide used	
			average number of application
Cereals	3,981	5,576	1.40
Potatoes	162	150	0.93
Sugar beet	206	475	2.31
Peas	91	77	0.85
Oilseed rape	124	198	1.60

From table 1.3 it can be seen that herbicides were used intensively on food crops especially on grain and tuber crops. In addition although these food crops are not necessarily the target of the herbicide treatment, it was reported that the drift of herbicide either by drops or vapour could also affect the agricultural crops (Norris,1953; Yates et al.,1977; Roberts,1982; Elliot and Wilson,1983).

As the usage of herbicide especially glyphosate has increased every year as mentioned earlier, drift problems are also increasing. Bearing in mind the large area of agricultural crops that have been treated by herbicide (table 1.3), there is a good case for an investigation being carried out to find the affect of glyphosate concentration on food crops. The background to the study is as follows :

1. Since the main target of glyphosate is rhizomes, tubers, roots or storage organs of plants because it is phloem translocated and its distribution pattern within the plants is the same as the photo-assimilates (Gouger and Geiger,1981; Dewey, 1982; Lund-Hoie,1983), potato, a well known and consumed tuber crop was considered suitable as a reference material to investigate such things and the physical drift effect. At the same time a method to determine the residue of glyphosate in this tuber crop was considered essential with the aim that the level of glyphosate in a tuber crop could be monitored quite easily and any

contamination caused by drift effect could be assessed.

This is particularly relevant to the potato growing area of Scotland where contamination of seed potatoes is a potential problem with the obvious implication for subsequent growth.

- ii. There are reports available showing that there was no adverse effect on cereal grain yield and its quality when glyphosate was applied at pre-harvest (O'Keefe, 1980, 1981a, 1981b). However this report was based on a bioassay technique for assessment. In addition no adverse effect does not necessarily mean that there was no glyphosate present in the grain (Lutman and Richardson, 1978).

There also was a report showing that there was a slight germination effect when grain was treated during pre-harvest with an application rate of 1.44 kg/ha (Sheppard et al., 1982) and Laermann and Lundehn (1980) reported that when poor weather conditions lead to the lodging of treated crops, glyphosate was adsorbed into the cereal husks. However they did not mention how they determined the glyphosate content in these grains.

It is believed that if the contact with glyphosate occurred during the stage where the grain still depends on the plant i.e. is still developing, then glyphosate will be translocated to the grains as its main storage organ because of the photo-assimilate distribution pattern.

When herbicide is applied to a large area of grain crops (table 1.3), the risk of drift cannot readily be avoided. There is the possibility of adverse effects due to glyphosate content in the grains. Therefore a method of determination of glyphosate in cereal grains should be developed, so that possible adverse effects could be monitored quite easily.

- iii. Another unique property of glyphosate when compared to other herbicides is that it exists in soluble form in any measured pH and its ionic form is pH dependent. In solution it can be neutral to three negatively charged. It was found that glyphosate could be deactivated by being bound strongly to the soil especially if the soil contains iron and aluminium (Sprankle et al., 1975; Hensley et al., 1978; McConnell and Hossner, 1985; Glass, 1987). Most of these authors proposed that this binding was due to complex formation between glyphosate and cations on the clay mineral in the soil. This is another specific behaviour of glyphosate.

In order to know or predict the fate of this herbicide in the environment, its affinity to form complexes with aluminium and iron(III) should be known. As had been predicted by earlier investigators, the affinity of glyphosate to form complexes with iron(III) and aluminium were very strong (Motekaitis and Martell, 1985). They determined the complex stabilities in nitrate

medium. They did not discuss their environmental implications. More information on these complex formations especially in other media including inert media should be determined to enable a better prediction or understanding of their chemical behaviour in the environment.

Although glyphosate is adsorbed strongly to the soil by aluminium and iron(III) which could reduce the environmental problem, adsorption or complex formation is often an equilibrium process, so this type of binding could be reversible (Calvet, 1980). Care should be taken not to shift the adsorption process in favour of releasing the glyphosate to the environment.

1.3 THESIS OBJECTIVES.

The main objectives of the thesis were related to the facts mentioned in section 1.2. Firstly to develop an improved method of determination of glyphosate residue in food crops. As can be seen from table 1.2, the method of detection was quite well established and with good sensitivity, so it was used without major modification. The same approach was also applied to the method of extraction. In the method of determination, the main difficulty lay with the clean-up procedures prior to analysis. Therefore these procedures were explored with the aim of achieving a significant reduction in time and materials required for sample clean-up or at least to

provide an alternative to present procedures. The compound obtained after clean-up by these procedures was subsequently determined by GC and HPLC.

For developing the clean-up procedures, two types of food crops, tuber (potato) and cereal/grain (barley) were used as a reference for the reasons mentioned in section 1.2. These two types of crops not only differ in their physical and chemical properties but their food storage organs also differ in the degree of exposure to the herbicide. One is relatively exposed because it is grown above the ground where the direct contact with chemical most probably happens (grain - barley) and the other is not exposed directly to the herbicide because it is grown below the ground (tuber - potato). An exception in the latter case is where the potato tubers in store come in direct contact with glyphosate treated straw. Claims of problems arising in this way have been made which can be best resolved by carrying out trace analysis work for glyphosate in suspect potato samples.

A satisfactory clean-up procedure could be applied to determine the actual amount of glyphosate in the glyphosate treated tubers (potato) and the relation to their physical symptoms could be obtained. These relationships could be used as a guide to assess the possibility of injuries by glyphosate drift or accidentally applied to the potato crop as mentioned above. The aim of developing a method of determination for glyphosate residues in barley (grain) was to use it for monitoring purposes because a large amount of herbicide was applied

to this crop area and many commercial organisations purchasing barley grain would wish the grain to be free of any chemical contamination such as glyphosate e.g. whisky distilling industry.

The other distinct property of glyphosate compared to other herbicides is that it can form complexes with metal ions. It has been reported that it can form complexes with iron and aluminium which are the major elements in the soil and these complexes are reversible. From this information the environmental implications should be considered.

To achieve the above objectives studies were performed as follows :

Chapter 2 : Analysis of glyphosate residue in tuber food crop (potato).

The purposes of the work in this chapter were to find the possibility of others clean-up procedures that have not yet been used in determination of glyphosate residue as in table 1.2 especially in potato. The advantages of these procedures were evaluated by using GC or HPLC. The main purpose of the study was to find a clean-up procedure that could be used in detection either by GC or HPLC, so that analysts will have a wider choice for their analysis. The advantages and the disadvantages of using GC and HPLC in analysis of glyphosate were evaluated and discussed. There are two methods of determination by HPLC, pre and post-column derivatization. Pre-column derivatization was preferred for various considerations.

Small cartridges of bonded-silica materials which could give a fast equilibrium and thus reduce the time of the clean-up process were tested. The suitability of the materials ranging from non polar to ion exchange either to retain glyphosate or the co-extractives were evaluated.

The ability of other materials especially the non polars such as cellulose, polyclar AT and activated carbon that have been applied to adsorb impurities from aqueous solution, to adsorb impurities from potato extract were also tested. This was done in order to get a relatively clean compound for detecting by fluorescence detector after chemical derivatization. The sensitivity of the procedures were also discussed.

Chapter 3 : Effect of glyphosate on food tubers (potato).

The aims of the work in this chapter were to investigate the effect of glyphosate on the tuber crop (potato) when the plant had been affected by this herbicide either by drift or if accidentally applied.

The glyphosate had been added to the tubers by spraying it on the plants at various rates of application during the pre-harvesting period.

The physical effects of this compound on the tubers were observed under normal storage conditions covering the period of seven months. During this period, the sprout length, number of eyes open and their resistance to disease or rotting were recorded at certain intervals. The contents of glyphosate in the tubers were determined by the method that had been developed in chapter 2.

From all the observations and determinations, relationships were drawn between the glyphosate rate of application and its effect on tubers physically and the glyphosate content in the tubers.

At the end of the storage period, the tubers subsequent growth performance was evaluated by growing them in the field. Their growth performance was recorded from time to time until the harvesting time and their yields were also recorded.

Chapter 4 : Analysis of glyphosate in cereal grain (barley).

In this chapter, the important consideration was to find a suitable analytical method for glyphosate residues in cereal grain by using barley as a reference. Emphasis was also given to clean-up procedure. Barley contains less anions and cations compared to potato, therefore a slight adaption to the method that had been evaluated in chapter 2 might be applied in this experiment. The suitability of Bond-Elut cartridges, both cation and anion were tested.

A recently used clean-up method, by employing its characteristic complexing property, using chelating resin was also employed to determine its effectiveness as clean-up material in this analysis.

Chapter 5 : Determination of complex stability constants
of aluminium and iron(III) with glyphosate and
their environmental implications.

This study was designed to obtain more information on glyphosate complexing affinity to trivalent metal ions; its complex stability constants with iron(III) and aluminium in inert perchlorate medium which was a different medium from what had been previously reported. More information on this parameter was needed in order to explain or to predict the fate of glyphosate in the environment.

From the above findings, glyphosate environmental implications were discussed.

Chapter 6 : Conclusions

The purpose of this chapter was to draw together an appreciation of the glyphosate situation where the implications of the above findings were discussed and to make some suggestions for further investigations.

CHAPTER 2.

ANALYSIS OF GLYPHOSATE RESIDUES IN FOOD

CROPS ESPECIALLY POTATO.

2.1 INTRODUCTION.

The potato tuber does not have direct contact with glyphosate which is normally foliar applied. Potato leaves are distorted or scorched when exposed to glyphosate at certain rates. Although the tubers do not show any visible effects as fast as the leaves, a certain amount of glyphosate may be translocated to the tubers.

The method of analysis for determination of glyphosate residues in various matrices including food crops has been mentioned in table 1.2 in chapter 1. The necessity for a simple method is also discussed in that chapter. The only method really tested for glyphosate residues in potatoes was a polarographic method at a fortification level of 2 mg/kg (Friedstad and Bronstad, 1985).

Maximum permissible levels of glyphosate in foods and crop commodities is generally 0.2 mg/kg in most foods for direct consumption (EPA, 1982b). In grain and grain products that are normally eaten in large quantities, the limit is lower (0.1 mg/kg). There is no maximum residue limit for potatoes mentioned.

The aim of the work in this chapter is to develop a simple method or at least to provide an alternative method of analysis for glyphosate residues in potatoes for the reasons given above.

The procedure adopted for the determination of glyphosate in potatoes or other crops normally involves three steps as detailed below :

- i. extraction
- ii. clean-up
- iii. detection

Firstly, a decision regarding the method of detection is essential before proceeding to the method of extraction and the method of clean-up.

There are many methods of detection of glyphosate or glyphosate residue in food crops such as:

- i. Gas chromatography after chemical derivatization.
- ii. High performance liquid chromatography using pre and post-column fluorogenic labelling.
- iii. Colorimetric methods.
- iv. Thin layer chromatography.
- v. Molecular emission cavity analysis
- vi. Polarographic methods.

With the above methods, mainly because of the sensitivity required, problems arise in quantitation of residues in crop extracts and also with regard to the availability of instruments and detectors. Particular attention was given to detection by gas chromatography

(GC) and high performance liquid chromatography (HPLC). These two instruments are widely used in pesticide residue analysis.

2.2 DETECTION OF GLYPHOSATE BY GAS CHROMATOGRAPHY (GC).

2.2.1 Introduction.

Detection of glyphosate by gas chromatography involves chemical derivatization. There is no method available yet to determine glyphosate by gas chromatography without any chemical derivatization. This is because of the nature of glyphosate which is not volatile, is very polar or ionic at high pH and very soluble in water. There is no stationary phase which could retain very polar or ionic nonderivatized glyphosate although there is a possibility of determining it by a Nitrogen-Phosphorous detector because its molecule contains N and P.

The several steps of derivatization required for glyphosate prior to detection by gas chromatography are as follows :

- i. 2 steps derivatization - acylation and methylation followed by detection by flame photometric detector (Monsanto Method, PAM 1977).

- ii. 2 steps derivatization - acylation and alkylation followed by detection by electron capture detector (Guinivan et al., 1985).
- iii. Single step derivatization. Derivatization at active hydrogens with N-methyl-N-(ter-butyl-dimethylsilyl)trifluoroacetamide followed by detection by flame photometric detector (Moye et al., 1984).
- iv. Simultaneous esterification and acylation with fluorinated alcohols - perfluorinated anhydrides followed by detection by flame photometric detector in phosphorus mode or electron capture detector (Deyrup et al., 1985).

Due to a limitation of available detectors, the only method tried was using an electron capture detector, i.e. methods ii and iv above.

It seemed that method iv was simpler compared to method ii. Method ii was developed to determine glyphosate in crop samples but method iv has yet to be tested on crop samples. Based on the simplicity of the derivatization procedure in method iv a decision was made to proceed with method iv for detection and quantitation of glyphosate residues in potato tubers.

2.2.2 Experimental.

Simultaneous esterification and acylation with fluorinated alcohols - perfluorinated anhydrides.

The method of detection by Deyrup et al., (1985) was adopted.

Instrumentation : A Pye Unicam GCD gas chromatograph equipped with ^{63}Ni electron capture detector at temperature 300°C was used for all measurements. A 1.5m x 2 mm i.d. silanized glass column was packed with 5 % SE 30 on gas chrom Q 100/200 mesh support. Oven temperature was 200°C for analysis of glyphosate. Carrier gas flow (N_2) was 25 ml/min.

Glassware and reagents : Reactions and dilutions were carried out in 5 ml reacti-vials (Pierce Chem.Co, U.S.A.) with teflon lined screw caps. Esterification and acylation reagents were trifluoro-ethanol and trifluoro - acetic anhydride. Glassware was soaked in methanolic KOH before use and was rinsed with deionized water. This was done as a precaution following a report by Deyrup et al., (1985) of glyphosate adsorption at low level to untreated glass.

Derivatization : A 10 ul volume of glyphosate standard (Greyhound, U.K, purity 97%) in water was added to a reacti-vial and water evaporated at 100°C with a stream of dry nitrogen. The tube was removed and allowed to cool to room temperature. 100 ul of trifluoro-acetic anhydride was added and 50 ul of trifluoro-ethanol was then added. The tubes were capped and heated at 100°C for 1 hour.

The reagents were removed at 25 °C with a stream of dry nitrogen, and the residue was dissolved in 20 ul of ethyl acetate, which had been stored over 4A molecular sieve, making it ready for GC analysis.

2.2.3 Results and discussion.

The electron capture detector is very sensitive. It needs extremely clean samples so that there are no interference peaks resulting from not using extremely pure reagents.

The retention time of the glyphosate derivative was ca. 1.7 minute. Even using standard glyphosate (dissolved in water) there were peaks of impurities close to this compound. The compound peak also had quite a high signal and formed as a tangent peak (see figure 2.1).

Because the retention time for glyphosate - TFE - TFAA derivative was quite short, if there were any impurities from the solvent and reagents they might interfere with the glyphosate derivative peak and quantitation of this compound was impossible. This effect could be seen clearly in figure 2.2, when standard glyphosate at pH 12 was retained on a Bond-Elut (Analytichem International, U.S.A.) SAX column, then eluted from the column by buffer of pH 2.0. The glyphosate derivative peak was covered by the impurities, making detection and quantitation for this compound very difficult or impossible.



Figure 2.1 Chromatogram of 0.5ng (0.5 μ l of 1ppm) glyphosate, after derivation.

Conditions :

Column : 1.5m x 2mm i.d.
Stationary Phase: 5% SE 30 on gas chrom Q 100/200 mesh support.
Carrier gas : N₂ at 25ml/min.
Oven temperature: 200 °C.
Detector : ECD at 300 °C.

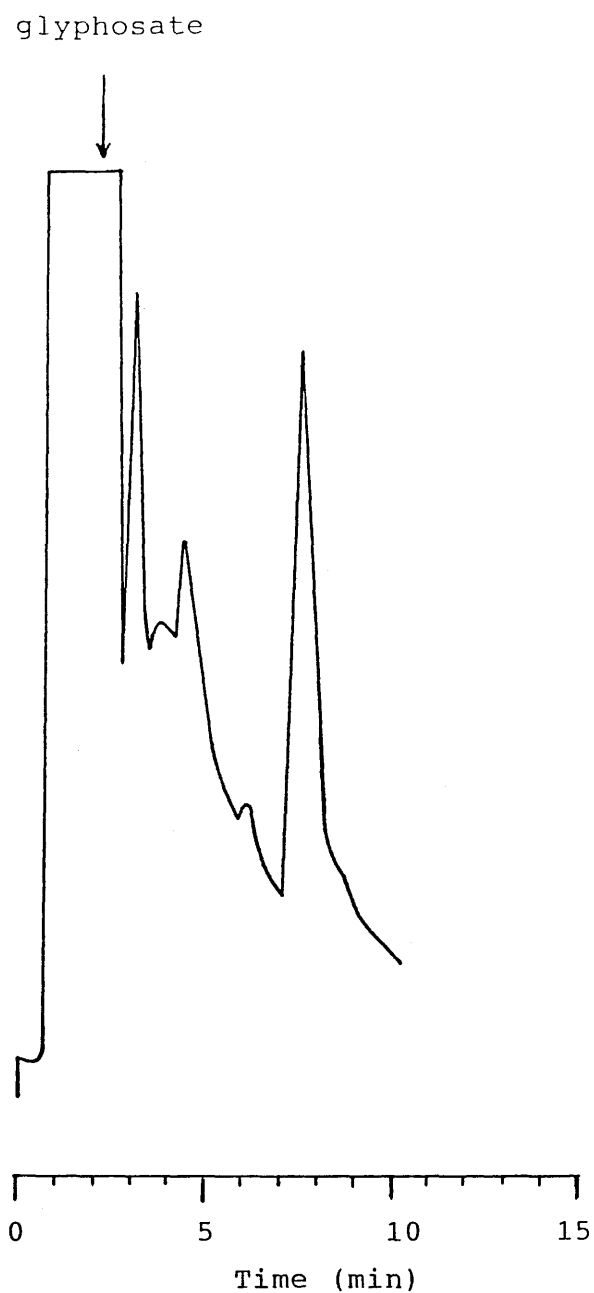


Figure 2.2 Chromatogram of standard glyphosate-derivative. Glyphosate was retained in Bond Elut SAX cartridge, then eluted by buffer pH 2.0. The glyphosate peak was covered by the impurities (RT~1.7 min).

Conditions : as for figure 2.1 .

Interference from the impurities could be reduced by increasing the retention time of this compound. In order to increase the retention time some parameters such as temperature, column length and flow rate of the carrier gas were manipulated. Reducing the temperature of the column did not result in sufficient improvement as the peak shape deteriorated and lost sensitivity (table 2.1). Flow rate of carrier gas could not be lowered down to less than 25 ml/min because the detector produced a very high base line signal and made it difficult for determination. Make up gas could be introduced to counter this problem.

The efficiency of the column can also be increased by increasing its length but at the same time this increased the analysis time (Bruner,1985). Doubling the length of the column will give a 1.4 x increase in resolution and twice the analysis time (Katz,1987). However, when a longer column was used, this also did not result in a significant increase in retention time. The reason why the longer column did not improve the retention time was that the longer column had a smaller diameter than the shorter one, so the amount of stationary phase in these two columns was not very different, resulting in similar retention times on the two columns.

Table 2.1 Effect of temperature on retention time and
peak height and shape of glyphosate -TFE-TFAA
derivative (column was 1.5m x 2mm i.d.).
5 % SE 30.

Sample injected	Oven temperature (^o C)	Retention time (min.)	Peak height/ shape
0.2 ul of	150	n.d.	n.d.
100 ppm	160	5.6	0.6 cm, quite
glyphosate			broad peak
standard	170	3.7	1.8 cm, quite
after			reasonable peak
derivati-	180	2.9	3.8 cm, quite
zation			good peak
	190	2.3	5.3 cm, good peak
	200	1.7	12.3 cm, very
			good peak
	205	1.4	13.4 cm, very
			good peak
	210	1.3	18.5 cm, very
			good peak

n.d. = not detected.

As the result of the unsuccessful detection and quantitation of a glyphosate derivative even using the glyphosate standard (retained on a Bond-Elut SAX cartridge, then eluted by buffer pH 2.0) it was decided to discontinue this method for the determination of glyphosate residue in potato because potato extracts would contain many more impurities than the glyphosate standard solution.

As a result of this lack of success with method iv, using gas chromatography with electron capture detector, attention was turned to the method of detection using high performance liquid chromatography (HPLC) as a part of the procedure to determine glyphosate residues in potatoes.

2.3 DETECTION OF GLYPHOSATE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC).

2.3.1 Introduction.

Detection of glyphosate by high performance liquid chromatography is quite similar to gas chromatography in the sense that it also involves chemical derivatization except for the determination in formulation and technical samples where the concentration was relatively high and did not require any derivatization for detection (Burnsand and Tomkins, 1979). There is no method available yet to determine glyphosate residue by HPLC without any chemical derivatization because of lack of sensitivity. The ionic, water soluble character of glyphosate makes analysis by HPLC advantageous over gas chromatography.

Also glyphosate is polar and water soluble and GC requires drying and then derivatization in organic solvent. HPLC derivatization is done in aqueous type medium so problems with completely anhydrous conditions which are difficult to achieve can be avoided completely.

Although glyphosate could not be measured at low levels by conventional detectors because it possesses very weak adsorptivity even at 200 nm and lack a of a conjugated system prevents it from fluorescing. Highly fluorescent derivatives could be formed and can be used either for pre-column or post-column procedures.

Although there are two procedures, pre and post-column, a pre-column was preferred for the following reasons :

- i. Although post-column procedures form derivatives on line they require more instrumentation and careful maintenance
- ii. Pre-column methods are simpler compared to post-column and require minimal equipment and analytical experience.
- iii. There is no continuous flow of derivatizing reagent(s) past the detector that may result in raised backgrounds (and therefore reduced sensitivity).

Pre-column procedure.

Bruton (1986) tested 4 reagents for pre-column derivatization namely dimethylamino naphthalene sulphonyl chloride (dansyl chloride), phenyl-isothiocyanate (PITC), o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloro-formate (FMOCCl) for fully automated amino acid analysis and he found that the best result was achieved with FMOCCl. Glyphosate carries a secondary amino group which could react with these 4 derivatization agents, so it could be expected that glyphosate can react with FMOCCl for optimum analysis as has been mentioned by Moye and St.John (1980). For pre-column derivatization, the method by Moye and St.John (1980) using FMOCCl was adopted.

2.3.2 Experimental.

9-fluorenylmethyl chloro-formate (FMOCCl) reacts via an S_N2 mechanism with amino nitrogen of both primary and secondary amines producing a carbamate having a fluorenyl group as the fluorophore.

0.1 ml of 10^{-7} - 10^{-3} M glyphosate was placed together with 0.9 ml of 0.025 M pH 9 sodium borate, 0.9 ml acetone and 0.1 ml of 10^{-2} M solution of FMOCCl in acetone, into a teflon capped 5 ml reacti-vial. The solutions were incubated at 23°C for 20 minutes. Three 1 ml portions of diethyl ether were used to wash away excess reagent. Appropriate dilutions were made with water before injection into the liquid chromatograph.

Detection by liquid chromatography.

A fluorometric HPLC was constructed from Waters Associated Model 6000 A pumps, a Rheodyne model sample injection valve equipped with a 20 ul sample loop and a Shimadzu Fluorescence HPLC detector model RF-530.

Chromatograms were recorded on a Servoscribe model RE 511.20 strip chart recorder and later were recorded on a Spectra Physic Sp 4290 integrator. Excitation was at 270 nm and emission at 315 nm. Isocratic operation was conducted at 1.0 ml/min with pH 4 phosphate buffer (0.1 M) containing 25 % acetonitrile by volume. Separations were achieved on an APS hypersil column (25 cm x 4 mm) (Shandon Southern, Runcon, England). Various solvent programmes and various columns were also attempted.

2.3.3 Results and discussion.

Results are summarized in table 2.2 and 2.3.

It was found that 0.1 M phosphate buffer with 25 % acetonitrile offered the best compromise between good sensitivity and a reasonable retention time. In anion exchange, retention usually could be increased by a decrease in ionic strength of the buffer and an increase in pH. On a silica-based stationary phase, a decrease in the percentage of organic modifier would also increase the retention time.

Table 2.2 Effect of different HPLC columns on separation
 and detection of glyphosate derivative.

Type of columns	Separation and detection
Vydax, anion exchange	Gave a broad peak
Ionosphere, anion exchange	Gave a very broad peak. Retention time quite long, about 23 min.
APS hypersil, anion exchange	Gave a sharp peak. Retention time ca. 13 min. Could detect easily 1 ng of glyphosate at attenuation 16.

Table 2.3 Effect of different acetonitrile percentages
 on peak shape.

% acetonitrile	peak shape
5	very broad
20	too broad
25	sharp peak
30	sharp peak but broader than 25 % acetonitrile.

By comparing the two methods, GC and HPLC, it was better to use an HPLC procedure over GC because the HPLC derivative gave a reasonably long retention time that could be manipulated with regard to the impurities peaks compared to the GC derivative which had a very short retention time. This criterion was useful in developing a new clean-up method.

When the method of detection of glyphosate standard was established, improvement of existing clean-up and extraction procedure could be dealt with.

2.4 EXTRACTION OF GLYPHOSATE RESIDUE.

To test whether the method of extraction was effective or not, a recovery test was carried out. The sample was spiked with a certain amount of glyphosate, then the method of extraction was evaluated.

There are many methods available for extraction of glyphosate residues from food crops which can be summarized as follows :

- i. Blending with mixtures of water and chloroform
(Moye and St.John,1980; Moye et al.,1983)
- ii. Refluxing with 0.25 M HCl (Archer and Stoke,1984)
- iii. Homogenising and sonicating with water (Guinivan et al., 1982)
- iv. Blending with a mixture of 0.1 M HCl and chloroform (Cowell et al., 1986).

Average recoveries of glyphosate obtained using the 4 methods listed above were good (> 80 %) (see table 1.2) and the decision to use method i was based on the following reasons :

- a. Only 2 of these methods had been tested on more than one crop (method i and iv) and an extraction method that could be applied to a number of crops was wanted.
- b. Method i has been used in a number of studies on glyphosate residue in crops so comparisons between the results of these studies and those described in this thesis would be simplified. Methods ii and iv used dilute HCl in an attempt to release glyphosate from possible conjugates with other plant constituents.
- c. Method iv was reported when recovery work using method i was almost completed and information suggested that the method was suitable for the purposes of the work described in this thesis. As the recovery and standard deviation reported for method iv did not seem to offer any improvement over method i, it was decided to use method i as the preferred extraction procedure in this thesis.

Extraction procedure.

25 g chopped potato, 25 ml chloroform and 50 ml water were placed in 500 ml stainless steel blender jar. Fortification of glyphosate was performed at this point for recovery. The mixture was blended at medium speed for 10 minutes. The jar was rinsed with 2 x 20 ml water and the content and rinses were placed in 250 ml centrifuge bottle. After centrifugation at 10,000 rpm for 20 minutes, the supernatant was filtered through Whatman No.1 filter paper into a 500 ml round bottom flask followed by 2 x 20 ml water which was used for rinsing the centrifuge bottle. Rotary evaporation was employed at a temperature below 40 °C to reduce the volume to about 5 ml and the extracts stored in the fridge for further treatment and analysis.

2.5 CLEAN-UP FOR GLYPHOSATE RESIDUE ANALYSIS.

2.5.1 Survey of current clean-up methods.

Special problems arose in the quantitation of glyphosate residues because the herbicide needs to be extracted from various matrices (crops, soil, etc.) with water as it defies extraction and any subsequent clean-up with organic solvents. Consequently, the difficulties and the main challenge lie essentially in clean-up. From the existing literature, it is known that the problem of determination of glyphosate residues rests mainly with the clean-up procedure. This information shows how important it is to simplify this procedure.

There are many clean-up procedures for the determination of glyphosate residues depending on what is the method of detection. For instance when detection is by GC the following clean-up treatments have been used :

- a. Using gel permeation chromatography, cation exchange column and liquid-liquid partition (Guinivan et al., 1982)
- b. Anion exchange column, charcoal treatment and cation exchange column (Monsanto method, PAM, 1977).

On the other hand in the case of HPLC, the detection method adopted in this work, the following clean-up treatments have been used :

- A. Pre-column labelling
 - a. using 190 g cation exchanger DOWEX 50W-X8, hydrogen form (Moye and St.John, 1980).
 - b. cation exchange resin (10 ml), Bio Rad AG 50W-X8 (Roseboom and Berkhoff, 1982).
- B. Post-column labelling.
 - a. using 190 g cation exchange resin, DOWEX 50W-X8, hydrogen form (Moye and St.John, 1980).
 - b. using 50 g cation exchange resin, DOWEX 50W-X8, hydrogen form (Moye et al., 1983)
 - c. using 15 ml cation exchange resin AG 50W-X8, hydrogen form and 15 ml anion exchange resin AG1-X4, chloride form (Archer and Stoke, 1984)
 - d. using 15 ml chelating resin Chelex 100 Fe (111) form, and 7 ml anion exchange resin AG1-X8, chloride form (Cowell et al., 1986).

As the aim of this work is to improve or to simplify the existing method, the first step must be to simplify the existing method of clean-up because of the reasons given previously. A recovery test was used in order to check the effectiveness of the clean-up procedure. For this purpose, the method of extraction and the method of detection were used as mentioned above in sections 2.4 and 2.3.2 using pre-column fluorogenic labelling.

2.5.2 An assessment of various Bond-Elut ion exchange cartridges in retaining glyphosate from standards and samples.

Bond-Elut cartridges contain bonded silica sorbent in which functional groups are covalently bonded to the silica substrate. Bonded silica sorbents are rigid materials that do not shrink or swell in different solvents, unlike many polystyrene based resins. For this reason, bonded silica equilibrates rapidly to new solvent conditions (Van Horne, 1985). Bond-Elut cartridges were tried as a clean-up material for the following reasons :

- i. Bond-Elut cartridges use less material. 100 mg or 500 mg per cartridge are used compared to existing procedures using 190 g cation exchange resin.
- ii. The time required for the clean-up procedure is significantly reduced because the solution volume is much smaller.

For testing the suitability of Bond-Elut cartridges for clean-up purposes, the cartridges of 100 mg sorbent and 1 ml volume were used.

The types of materials (sorbent) used for evaluation could be polar, cation or anion exchange. These sorbents were chosen because water, a polar solvent, was used to extract glyphosate from the crop matrix.

From the above literature, most analysts used cation and anion exchange resins. Some analysts like Moye and St. John, (1980) and Roseboom and Berkhoff, (1982) used only cation exchange resin for this purpose. Cowell et al., (1986) used chelating resin as a clean-up material.

In this work, cation and anion exchange Bond-Elut cartridges were evaluated as clean-up materials. Before these Bond-Elut cartridges could be evaluated for sample extracts, firstly they had to be tested with glyphosate standard.

Evaluation of Bond-Elut cartridges with glyphosate standard.

Standard preparation : 0.1034 g glyphosate was dissolved in 100 ml mixture of deionised water (95 % v/v) - methanol to yield 1000 ug/ml stock solution. Methanol was added as a fungal growth inhibitor. The lower concentrations of standards were made by diluting this stock solution with water. All the standard solutions were refrigerated.

The Bond-Elut sorbent is stable within a pH range of approximately 2 to 7.5. Above pH 7.5 the silica substrate is susceptible to dissolution in aqueous solution. Below pH 2.0 the silyl linkage is labile and the functional groups on the surface begin to cleave, changing the sorptive properties in a non-reproducible fashion. Nonetheless, in practice, bonded silica may be used for sorbent extraction in a pH range of 1 to 14, since degradation of the sorbent is a finite process and sorbents are usually exposed to solvent for only short periods of time.

In the above existing procedures of clean-up, sample extracts are adjusted to a certain pH before passing through the resin columns. These include adjusting to pH 1 (Moye and St.John, 1980) or to pH 2 or less (Moye and St.John, 1983; Cowell et al., 1986) before passing through cation exchange or chelating resin or adjusting to pH 10 (Archer and Stoke, 1984) before passing through anion exchange resin.

The idea of using Bond-Elut cartridges for clean-up purposes is that in solution, glyphosate exists in ionic form. By manipulating the pH, the ionic strength of glyphosate could be increased or decreased. This ionic form of glyphosate could bind to the sorbent by an ion exchange mechanism. By manipulating the pH it seemed possible for glyphosate to be retained strongly on the ion exchange column, while the impurities could be washed away by passing through water, leaving clean glyphosate on the column. After this the clean glyphosate could be removed

from the column with a specific solvent before proceeding to the determination.

Using cation exchange Bond-Elut cartridge, SCX.

The functional group of this sorbent is propylbenzene- sulphonyl, hydrogen form, a strong cation exchanger with the capacity of 100 mg sorbent cartridge being 0.12 meq.

By considering all above factors, the pH of glyphosate standard was adjusted to pH 2 with a buffer solution.

Procedure : The SCX cartridge was washed with one volume of methanol, to wet or to activate the sorbent. Then the excess methanol was removed by passing through one volume of deionised water. One ml of glyphosate standard was passed through the cartridge and the standard eluate collected. The column was then rinsed with 1 ml deionized water and the water eluate was collected. Finally, the column was eluted with buffer pH 4.0. The buffer eluate was collected.

Glyphosate content in all above eluates were determined by HPLC as follows:

To every 1 ml of eluate, approximate 0.1 g K_2CO_3 was added with shaking to bring the pH to 11. 4 ml of H_2O and 5 ml 0.01 M FMOCCl in acetone were added to the tube which was then capped and reacted at $23^{\circ}C$ for 20 minutes. The reaction mixture was washed 3 times with 5 ml of diethyl ether, diluted to 10 ml with H_2O and injected onto the HPLC. Column comparisons were made with standards at the

same pH which were similarly derivatized.

For above procedure, the following steps were applied.

- i. All the eluates of above process were collected in teflon capped bottles.
- ii. The flow rate of the solution passing through the column was adjusted to about 0.8 - 1.0 ml/min by applying pressure with a pipette bulb.

Results and discussion.

Results are presented in table 2.4.

Table 2.4 Performance of Bond-Elut cation exchange cartridge, SCX to retain glyphosate at pH 2.

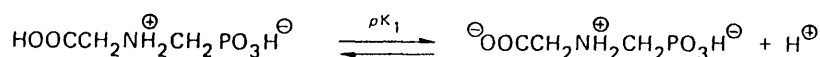
Glyphosate standard	% not retained at all by the cartridge	% eluted by H ₂ O washing the cartridge	% retained on the cartridge
in buffer			
pH 2	79.8	19.9	0

All figures the mean of two replicates.

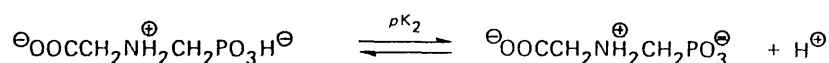
It was found that when the pH of the standard glyphosate was adjusted to 2, there was 0 % glyphosate retained on the cartridge. Based on this result, it was not necessary to try the glyphosate standard adjusted to a pH less than 2 or 1, as was the case with some analysts

using cation exchange resin.

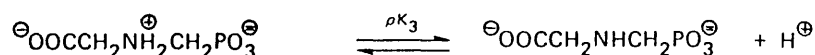
The results of the above experiment could be explained by looking at the properties of glyphosate. Glyphosate has three functional groups and its properties depend on the pH of the medium.



Monoanion

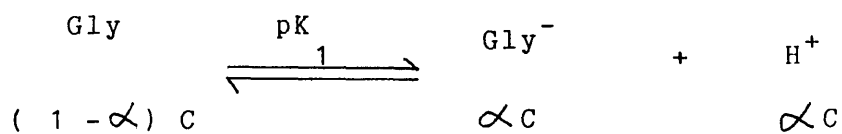


Dianion



Trianion

Values for pK_1 , pK_2 and pK_3 have been reported (Madsen et al., 1978) to be 2.27, 5.58 and 10.25 respectively.



$$10^{-pK_1} = \frac{[\text{Gly}^-][\text{H}^+]}{[\text{Gly}]} = 10^{-2.27}$$

$$\begin{array}{lcl} \text{at pH} = 2, & [\text{H}^+] = 10^{-2} \text{ M} & \longrightarrow \alpha = 0.35 \\ \text{at pH} = 1, & \alpha = 0.05 & \end{array}$$

At pH 2, 35 % of the glyphosate exists in mono anionic form and 65 % in neutral form. At pH 1 it was calculated that 95 % glyphosate would be in neutral form and 5 % only in mono anionic form.

At pH 2 only 35 % glyphosate is in mono anionic form and there is no glyphosate in any cationic form. This phenomenon could explain why it was retained quite loosely on the cartridge at this pH; secondary interaction with this polar sorbent and also by binding with the cationic portion of the sorbent meant that it could be easily washed out by water washing. At pH 1 there was no glyphosate in cationic form that could exchange with the sorbent, and therefore be retained strongly by the cartridge. Hence, as a test had been carried out by other analysts at pH 1 using cation exchange resin it was not considered necessary to repeat it here.

Although preliminary measurements showed that glyphosate should be able to take up a proton to form a positive ion that could exchange with cation exchange sorbent, the corresponding pK value is negative (Madsen et al., 1978). Hence this possibility was neglected because it could happen only at an extremely low pH in which the Bond-Elut sorbent is not stable.

From the above results and compared with several results done by others using cation exchange resin, it was decided not to use Bond-Elut cation exchange cartridges as a clean-up material because they could not retain glyphosate strongly.

By looking at the structure of glyphosate, the molecular charge could be increased by increasing the pH. In any measured pH (1 - 14), glyphosate exists in anionic form. This property could enable glyphosate to be retained on an anion exchange cartridge rather than on a cation exchange cartridge.

Using Anion Exchange Bond Elut Cartridge, SAX.

After failing to get successful results by using a cation exchange Bond-Elut cartridge, an anion exchange Bond-Elut, SAX was used instead to evaluate its performance for retaining glyphosate.

The functional group of this sorbent is trimethylaminopropyl which is a strong anion exchanger in the chloride form. The capacity of 100 mg is 0.12 meq.

By manipulating the pH of the medium, glyphosate could exist at different anionic strengths. Glyphosate is monoanionic at pH 4, dianionic at pH 8, and trianionic at pH 12. Glyphosate exchanging power will vary with pH.

Procedure : The cartridge was washed with one volume of methanol, then it was rinsed with one volume of deionized water to remove excess methanol. Glyphosate standard at a set pH was passed through the column. Eluate from the column was collected. Then the cartridge was rinsed with 1 ml of deionized water and this water eluate was collected. Finally the cartridge was eluted with buffer solution of a certain pH. Different fractions of eluate were collected. Glyphosate content in all above eluates was determined by HPLC using the same procedure as

mentioned above in the section involving cation exchange Bond-Elut cartridge.

Results and discussion.

The results for the influence of pH on the retention of glyphosate standards and the efficiency of eluent solutions of different pHs to elute glyphosate from a Bond-Elut anion exchange cartridge can be seen in table 2.5.

At pH 5.5 about 50 % of glyphosate is in monoanionic form and 50 % is in dianionic form. At this pH there was quite a substantial amount of anion that could exchange with anion exchange sorbent but it showed that it did not have enough anionic strength to fully exchange with the sorbent and hence be retained strongly. At this pH about 10 % of glyphosate was strongly retained by the cartridge, 23 % was retained quite loosely, i.e. could be washed away by water washing and about 67 % could not be retained at all by the cartridge.

Based on the above findings, the pH of glyphosate was adjusted to 12; in this condition glyphosate exists in the trianionic form. At this pH all functional groups of glyphosate become ionized and glyphosate has a strong anionic character that could be fully exchanged with sorbent and hence be held strongly by the cartridge.

When glyphosate was retained strongly on the cartridge (at pH 12), i.e. it was not eluted by water, the impurities could be washed out from the cartridge by passing through water several times. Then glyphosate

Table 2.5 Influence of pH in retaining glyphosate standard and efficiency of eluent solutions of different pHs in eluting glyphosate from bond-Elut Anion Exchange Columns.

No.	Glyphosate standard	Eluent solution	% Glyphosate ^a						
			in glyphosate standard eluate (not retained at all by the column)	in water washing eluate (retained quite loosely by the column)	in collected fractions (ml) (retained strongly by the column)	0-1	1-2	2-3	3-4
1.	Buffer pH 5.5	Buffer pH 2.0	67	23	10	-	-	-	
2.	Buffer pH 12	Solution pH 2.0	-	-	-	-	-	-	
3.	Buffer pH 12	Buffer pH 2.0	-	-	5	90	5	-	
4.	Buffer pH 12	Buffer pH 1.0	-	-	100	-	-	-	

a = average of duplicates.

could be removed from the cartridge with a lower pH solution and a clean glyphosate preparation collected.

From table 2.5 it was found that glyphosate could be retained strongly on a Bond-Elut anion exchange cartridge. Glyphosate was removed from the column by using buffer pH 1. When using an elution solution of higher than pH 1, more elution volume was needed.

Glyphosate at pH values between 5.5 and 12 was not investigated because it was essential to have the most favourable conditions for glyphosate to be retained on the cartridge in the sense that it would not be washed out by applying several lots of water in the process of removing the impurities. Also some of the buffers used between these two pH values contain amino^{and phosphate} groups which could interfere with the determination of glyphosate where they could react with the derivatizing agent.

Evaluation of Bond-Elut cartridge for glyphosate treated sample.

After the glyphosate standard had been successfully isolated by using Bond-Elut anion exchange cartridges as mentioned above, this cartridge was used to evaluate the isolation of glyphosate from glyphosate spiked potatoes.

Procedure :

- i. Preliminary treatments for the anion exchange cartridge before it was ready to accept sample were as mentioned earlier.
- ii. Extraction procedure and volume reduction for the potato extract were as mentioned above. The extract was adjusted to pH 12 firstly with 10 M and

finally with 1 M KOH and the final volume was made to 25 ml. It was then filtered through Whatman No. 1 filter paper. One ml of the extract was taken and passed through the cartridge. Sample eluate was collected, then the cartridge was washed with 1 ml water and the water eluate collected. Finally, the cartridge was eluted with 1 ml buffer pH 1 and the eluate was collected. To every eluate 0.15g K_2CO_3 was added to raise the pH to 11. Other procedures prior to injection into the HPLC were as mentioned above.

Results and discussion.

The performance of Bond-Elut anion exchange cartridges for retaining or isolating glyphosate from spiked potato samples could be seen in table 2.6.

Table 2.6 Performance of Bond-Elut anion exchange cartridge on retaining glyphosate from glyphosate spiked potato.

Sample pH	% glyphosate ^a				
	in sample eluate(not retained at all by the cartridge)	in water washings (retained quite loosely by the cartridge)	in collected eluate		
			0-1	1-2	2-3 ml

12	80 %	20 %	-	-	-
----	------	------	---	---	---

a - mean of duplicates

It was found that the Bond-Elut anion exchange cartridge lost its ability to retain glyphosate from the potato extract sample. It seemed that only a certain amount (about 20 %) of glyphosate at pH 12 in potato extract was loosely retained and the rest was not retained at all by the cartridge, although at this pH, a glyphosate standard in deionized water was strongly retained on the cartridge. Retention of glyphosate in potato extracts at pH 12 seemed almost the same as the glyphosate standard in water at pH 5.5 (see table 2.4).

In explaining why the Bond-Elut anion exchange cartridge was not effective in isolating or retaining glyphosate from the potato extract compared to glyphosate in water, there are many possibilities some of which are mentioned below :

1. Glyphosate could react with metal cations in the potato extract (e.g. Mg^{2+} , Fe^{3+} , Zn^{2+} , etc) to form complexes. Madsen et al., (1978) have reported that glyphosate could form stable complexes with metal ions such as Cu^{2+} , Zn^{2+} , Mn^{2+} , Ca^{2+} , and Mg^{2+} . The formed complex compounds have less anionic charges compared to glyphosate itself and consequently these glyphosate metal ion complexes would be retained less by the cartridge. For example : $Mg^{2+} + L^{-}$. Mg-glyphosate complex has one anionic charge compared to glyphosate which has 3 anionic charges.

2. The potato extract contains anions for which the sorbent has an extremely high affinity. As a result, glyphosate would be less retained by the cartridge (sorbent).

3. The potato extract has a high ionic strength ($> 0.1 \text{ M}$). The high concentration of anions in the extract would compete with anionic glyphosate for the sorbent, promoting elution of the anionic glyphosate (Van Horne, 1985). To check whether the metal ions affected the glyphosate retained on the column, a potato extract sample was adjusted to pH 14 with the aim of reducing the solubility of most of the mineral ions and hence reducing the formation of glyphosate-metal ion complexes. All other treatments performed on the sample were similar to the sample being adjusted to pH 12. The result showed that the retention of glyphosate on the Bond-Elut anion exchange cartridge was almost the same, whether it was adjusted to pH 12 or 14. This observation showed that metals ions appeared to have no significant influence on retaining glyphosate from potato extract by the cartridge. If metal ions were involved in the effect, the cartridge should have retained more glyphosate at pH 14 than at pH 12.

In the case of the second possibility, viz. the effect of high selectivity anions, the Handbook of Sorbent Extraction Technology (Van Horne, 1985) lists citrate ions as having relatively a high anion selectivity to anion exchange sorbent compared to other anions. Citric acid exists as citrate ion at high pH as in the potato extract

when it is introduced to the cartridge. The content in potato has been reported to be between 0.5 -1 % (Smith, 1968).

It is possible that the high content of citric acid could affect glyphosate retention by Bond-Elut column and, therefore, the effect of citric acid on the retention of glyphosate on Bond-Elut anion exchange cartridges was evaluated.

The citric acid content of the potato was assumed to be ca. 0.75 % (average of range reported). From the analytical procedure, this worked out about 8.0 mg citric acid per ml in the final solution. Standard glyphosate in 8.0 mg citric acid/ml was made up. The pH of this solution was adjusted to 12, and 1 ml of the solution was passed through the Bond-Elut anion exchange cartridge. The glyphosate content of each fraction was determined as described earlier.

The results were recorded in table 2.7.

Table 2.7 Effect of citric acid (8 mg/ml) on the retention of glyphosate on Bond-Elut anion exchange cartridge.

% Glyphosate ^a					
Solution pH	in standard solution eluate (not retained at all by the cartridge)	in water washings (retained quite loosely by the cartridge)	in collected eluate		
			0-1	1-2	2-3 ml
12	69 %	27 %	-	-	-

a = mean of duplicates

It seemed that the pattern of glyphosate retained in the cartridge was similar to that obtained with glyphosate in potato extract. Less of the glyphosate in potato extract was retained quite loosely in the cartridge than in the made up citric acid solution. This could be because potato extract contained more than 0.75 % citric acid and also because it contained quite substantial amounts of amino acids and organic acids (Smith, 1968) that could form anions at higher pH.

A third possibility was explored. Citric acid is a major constituent of organic acids in potato tubers. Other organic and amino acids are present in lower amounts. Based on the earlier assumption of ca. 0.75 % citric acid in the potato this resulted in about 0.04 M in

final potato extract solution. The actual ionic strength of the potato extract was not determined.

It seems possible that the citrate ion and other anions could compete with glyphosate retained on the cartridge because of their high selectivity and their presence in relatively high concentrations compared to spiked glyphosate where it was noticeably absent (< 10 ppm).

To reduce these competing anions, it would be possible to use Bond-Elut exchange cartridges in series so that each cartridge would retain some of these competing ions and the last cartridge would receive a smaller concentration of these anions, hence glyphosate could be retained due to less competition.

1,2,3,4 and 6 Bond-Elut anion exchange cartridges were tried in series to retain glyphosate from standard glyphosate solution in 8.0 mg/ml citric acid. All standard solutions and eluates were adjusted to pH 12 before introducing to the cartridge.

Results can be seen in table 2.8.

Table 2.8 Effect of using multiple Bond-Elut anion exchange cartridges to retain glyphosate from glyphosate standard in 8.0 mg/ml citric acid

% Glyphosate					
Anion exchange cartridge used	in standard solution (not retained at all by the cartridge)	in H ₂ O washings (loosely retained by the cartridge)	in collected eluate 0-1 1-2 ml		
1	69 % ^a	27 % ^a	-	-	
2	96 % ^a	-	-	-	
3	96 %	-	-	-	
4	96 %	-	-	-	
6	96 %	-	-	-	

a - average of duplicates

The above results showed that using one or more Bond-Elut anion exchange cartridges were totally ineffective in isolating glyphosate from glyphosate standard solution containing 0.8 mg/ml citric acid. The result also showed that the first cartridge could loosely retain about 27 % glyphosate while the second, third, fourth and sixth cartridge could not retain any. If

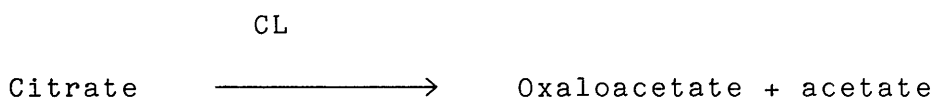
competing anions have a major influence on glyphosate retention, then logically glyphosate should be retained in the cartridges in order : sixth cartridge > fifth > fourth > third > second > first cartridge, as less and less competitive anions are introduced to the next cartridge because a proportion of each was retained on the earlier ones.

Complicating the procedure were the following observations :

1. Every time the solution was passed through to the next column, it was diluted by adding washing solution from the pH electrode and the reacti-vial. This treatment lowered the ionic strength of the ions but at the same time the activity of anions of organic and amino acids were increased, compared to glyphosate which was at a low level (spiked at 10 ppm).
2. Before the solution was passed through the cartridge, its pH was adjusted to pH 12. The washing solution from the electrode and tube was combined with the test solution and then passed through the cartridge. This process was repeated before the solution was passed through the next cartridge. This means that each cartridge would receive more solution than the earlier one. The first cartridge received 1 ml of solution, the second one received about 2.5 ml, the third one received 3.5 ml, the fourth one received 4.0 ml, the fifth one received 4.5 ml and the sixth cartridge received about 5.0 ml.

The cartridge sorbent was stable at $2.0 < \text{pH} < 7.5$. It was also stable at lower or higher pH if it was exposed to this solution for a short period of time. When a bigger volume was used, the column was exposed for a longer time. To receive a sample at pH 12, the silica substrate of the sorbent was susceptible to dissolution if this solution passed through the cartridge over a relatively long time (approximately 5 minutes). Dissolution made the cartridge lose its capacity to retain the compound.

As citric acid could have such an influence on glyphosate retention it was decided that a method to remove or destroy the citric acid in the solution had to be found. There is an enzyme that can destroy or convert citric acid, citric lyase (CL) as follows (Boehringer, 1977/88) :



It is unlikely that an enzymic method to destroy citric acid will solve the problem of glyphosate retention on the Bond-Elut anion exchange cartridge for the following reasons :

- i. Citric acid content in the potato extract is about 187.5 mg in total with the assumption that its content is ca. 0.75 %. Large amounts of citric lyase would be needed to destroy the citric acid in the sample.

ii. This process produces two molecules of acid from one molecule of citric acid. As a result there would be a higher salt content at high pH and these anions could still result in a competitive problem for glyphosate retention on the cartridge.

There was also the possibility of diluting the potato extract in order to reduce the salt or ionic strength effect but this option would significantly reduce the sensitivity of glyphosate quantitation coupled with the other problem that the citrate ion would still be in the dilute extract in the same ratio competing with glyphosate for the sorbent.

Although both factors, high selectivity anions and high ionic strength play an important role in retaining glyphosate in potato extract to the Bond-Elut anion exchange cartridge, it would seem, from the above observations that the high selectivity anions would seem to have the greatest influence.

It would thus appear that to clean-up potato extract through a Bond-Elut cartridge neither cation nor anion exchange alone would be effective. Hence Bond-Elut cartridges have to be ruled out as a simple clean-up material in the determination of glyphosate residues in potato.

As a result of the failure of using Bond-Elut cartridges, other materials or compounds were investigated to improve or to simplify the clean-up procedure for the determination of glyphosate residues in potatoes.

2.5.3 The use of various Bond-Elut cartridges and other materials in retaining co-extractives from samples.

After failing to retain or isolate glyphosate in potato extracts on the Bond-Elut cartridges, the reverse procedure of retaining the impurities on the cartridges was tried instead. In this process co-extractives in potato extracts could be retained in the cartridges while the glyphosate compound could pass through without any retention.

For this purpose, 4 types of Bond -Elut cartridges, SAX - anion exchange, SCX - cation exchange, C18 - octadecyl, non polar and NH_2 - aminopropyl, polar were tested to see whether they could retain the impurities present in the potato extract.

Procedure : 1 ml of potato extract at certain pH was passed through all the tested cartridges. The eluates were collected and the glyphosate content in these eluates were determined by HPLC using pre-column fluorogenic labelling as mentioned earlier.

Results: From the chromatograms, it was found that all tested Bond-Elut cartridges were ineffective in retaining impurities in potato extracts for glyphosate residue determination. Chromatograms of these 4 cartridges were almost the same and were not much different from the chromatogram of the extract without passing through any cartridge.

Based on above experiments Bond-Elut cartridges could not be used as a clean-up material either to retain the compound or to retain the co-extractives.

Using various materials or compounds to retain potato extract co-extractives.

Some materials or compounds especially non polar ones were explored for their suitability to retain potato extract impurities but at the same time allow glyphosate to pass through without any retention. In this study, all materials were made up for column chromatography and 5 g of each material was used.

Materials or compounds tried were - activated carbon (powder and semi coarse form), cellulose, DEAE cellulose and polyclar AT. These compounds were tested because of their uses as detailed in table 2.9.

Table 2.9 Background to compounds tested to retain
co-extractives from potato extract.

Compound	usage
Activated carbon	adsorbs colour and odour from aqueous solutions (Norit,U.K).
cellulose	a mixture with activated carbon has a high capacity for retaining plant pigments (Lawrence,1981).
Polyclar AT	adsorbs phenols and tannin from aqueous extract (Loomis and Betteile, 1966)
DEAE-cellulose	separates proteins and nucleic acids (BDH, 1987/88).

Procedure : 10mg/kg glyphosate spiked potato extract at pH 12 was used as a test solution where 1 ml portions of this solution were passed through the chromatographic columns of the materials tested. Water was used as eluent. Several fractions of eluate were collected and glyphosate content in these eluates were determined by HPLC pre-column fluorogenic labelling as mentioned earlier.

Results and discussion.

Results for above attempts are given in table 2.10.

Table 2.10 Performance of some adsorbents in retaining
potato extract co-extractives.

Type of column	recovery (total)	impurities interference
Semi coarse, activated carbon	55 %	not much interference
Cellulose	0	same as above
Cellulose DEAE	0	same as above
Mixture of activated carbon, powder + cellulose (1:1)	0	same as above
Polyclar AT	d.q.	a lot of interference
Mixture of activated carbon, powder and cellulose DEAE (1:1)	55 %	not so much interference
d.q. : difficult to quantify		

To explain the behaviour on retaining or adsorbing glyphosate, a simple test was carried out. The pH of these materials was determined by weighing out 2 g of each material which was shaken in 25 ml water and the pH determined. It was found that the pH of powder and semi coarse activated carbon was 10.5, cellulose was 4.6, DEAE cellulose was 5.4 and polyclar AT was 9.6.

At pH 12 glyphosate exists in three anionic forms which would be very difficult to retain using a non polar compound.

From these observations it could be explained that one of the reasons why these materials adsorbed glyphosate differently was because they were quite acidic in character. This could reduce the anionic strength of glyphosate through ionic reaction and increase the adsorption of glyphosate onto these materials. However the properties of these materials played an important role.

The above results also showed that of materials tested, only activated carbon potentially could be used as a clean-up material for the determination of glyphosate residues in potatoes. So activated carbon was studied further for its suitability as a clean-up material.

2.5.4 The use of activated carbon in retaining co-extractives from samples.

2.5.4.1 Introduction.

Activated carbon is known to adsorb some materials such as colours from molasses solution, odours in liquor (Norit,U.K) and impurities from water (Perrich,1981). Activated carbon can also adsorb a lot of organic compounds to various degrees and their solubility has been presented (Ford,1981). If the solubility of the adsorbate is increased the adsorption to carbon will decrease. Polar groups which have a high affinity for water usually diminish adsorption from aqueous solution. Low pH promotes the adsorption of organic acids whereas a high pH favours the adsorption of organic bases. The forces that make activated carbon adsorb constituents are weak Van Der Waals and the pore structure also plays an important part in this adsorption (Ford,1981).

Charcoal had been previously used as one of the clean-up steps in the determination of glyphosate by gas chromatography (PAM,1977). Charcoal column chromatography was used for separation of saccharides according to the degree of polymerization into monosaccharides, dissacharides, trisaccharides and so on (Whistler and Muller,1962).

From above information and findings, activated carbon could be used as a clean-up material to adsorb the impurities from potato extract and let the glyphosate pass through because of its ionization properties. Activated carbon column chromatography seemed a reasonable approach for this purpose rather than by shaking glyphosate potato extract with activated carbon and then filtering because the interference of the impurities could be reduced by controlling the volume of eluent collected.

In liquid phase application, the choice between powdered and granular is complicated by the fact that often either method could be effective. For example, in the purification of water supplies, some water works are based on powdered carbon and others on a granular carbon. This also happens in the sugar and glucose industries where both powdered and granular carbon-based refineries are in existence (Norit, U.K.). A test had to be carried out to fully assess the clean-up potential of these different size grades of activated carbons.

2.5.4.2 Material and method .

For this purpose, activated carbon, powdered form, Ultrasorb and semi coarse form obtained from Chemviron, Belgium were used for evaluating the effectiveness of activated carbon as a clean-up material.

2.5.4.3 Preliminary evaluation.

To reduce the effect of adsorption or binding of carbon to glyphosate by mineral ions especially heavy metals such as Fe^{3+} , Zn^{2+} , Al^{3+} , Mn^{2+} etc. which could form complexes with glyphosate, activated carbon was soaked for 2 hours in concentrated HCl. The excess HCl was removed by washing with deionized water and then with slightly alkaline deionized water until it was neutral. Then it was dried in the oven, cooled and stored in a tightly sealed container.

For carbon chromatography, a 1 cm diameter column containing 5 g activated carbon was used. Before receiving the sample, the column was equilibrated with a H_2O -solution at pH 11. The elution solvent was H_2O solution pH 11. Flow rate was increased by water suction.

For this evaluation, a 10 ppm glyphosate spiked potato extract at pH 11 was used.

pH 11 was chosen for sample and eluent solution because at this pH about 85 % of the glyphosate is present in trianionic form. It could pass through the column without retention because of the non polar character of activated carbon. At this pH all the eluates could be used directly i.e. without any pH adjustment derivatization could be carried out prior to HPLC analysis.

5 ml fractions of eluent were collected. Glyphosate content and effect of impurities in this solution were determined by HPLC pre-column fluorogenic labelling as mentioned earlier.

Various activated carbon columns were set up for checking their performance to isolate impurities and the recoveries of 10mg/kg glyphosate spiked potato samples were attempted. In this experiment inert material - medium size sand, was used in a mixture with the above adsorbents to increase the flow rate of the column. Results are given in table 2.11.

Table 2.11 The performance of various activated carbon columns in recovery test with 10 mg/kg glyphosate spiked potato.

Material used	Recovery (total)	Impurities interference
powder C:semi coarse C : sand, 1 : 3: 2	56 %	clean eluate
powder C:semi coarse C 1 : 7	41 %	clean eluate
powder C:sand, 3 : 7	31 %	interference on compound peak
semi coarse C	32 %	clean eluate
powder C:semi coarse C 1 : 3	62 %	clean eluate

The results showed that neither powdered activated carbon nor semi-coarse activated carbon alone were effective as clean-up materials for the determination of glyphosate in potato extracts. Powdered or semi coarse activated carbon alone could adsorb glyphosate stronger

than the mixture of these materials. However a mixture of these two types of activated carbons in a certain ratio showed promise as a clean up material. This phenomenon could be explained by looking at the properties of activated carbon.

The surface area of activated carbon generally ranges from 450 - 1800 m²/g with a pore volume of 0.7 to 1.8 ml/g for both finely ground and granular. This is due to the fact that most of their surface area lies in their vast internal pore structures. However only a portion of this area and pore volume (pores of the proper size) will be available for adsorption.

Therefore the total surface area and pore volume data should not be used to rate the probable effectiveness of an activated carbon. An activated carbon with an extremely high surface area may adsorb very rapidly but its adsorptive capacity could be low because it lacks pore volume to hold the adsorbed material (Hutchins, 1981).

The effect of grinding activated carbon material is negligible, provided sufficient time is given for the carbon to reach its equilibrium adsorption value (Norit, U.K). One would imagine that a finely ground material would have a much larger surface area than the equivalent weight of coarser material. This is not necessarily the case. Surface measurement techniques show that a fine powder of a non porous material like sand with an average particle size of 20 um diameter has a surface area of only 0.5 m²/g.

One of the reasons why the recovery was not so high was water solution at pH 11 when used as an eluent could not act as an effective buffer solution. It was subject to pH change. Base on these results work to improve the recovery was carried out as below :

- i. Buffer pH at 11 was used to replace the water solution pH at 11 as eluent. This would give better control for glyphosate passing through the column.
For this purpose K_2CO_3 10 g/litre was used.
- ii. More volume of sample was introduced through the column in the hope that it would reduce the effect of glyphosate adsorption by the column.

Results for the above attempts are given in table 2.12.

Table 2.12 Effect of using buffer pH 11 as eluent and introducing more sample volume on recovery of glyphosate spiked potato.

Material used	weight of material	volume of sample	recovery %
powder C:semi coarse C 1 : 3	5 g	1 x 1 ml of 10 ppm	80.1
powder C:semi coarse C 1 : 3	5 g	1 x 1 ml of 0.1 ppm	0
powder C:semi coarse C 1 : 3	6 g	1 x 1 ml of 0.1 ppm	0
powder C:semi coarse C 1 : 2	5 g	1 x 1 ml of 10 ppm	98.1
powder C:semi coarse C 1 : 3	5 g	3 x 1 ml of 10 ppm	93.8

It was found that by using buffer pH 11 as a replacement for water solution pH 11, the recovery was significantly improved, from about 60 % to more than 80 %. Using more sample volume could also increase the recovery. The mixture of fine and semi-coarse activated carbon at ratio 1:2 gave the best result in terms of recovery (using 10 ppm spiked sample). The ratio 1:1 for these two grades of activated carbons could not be tested because the flow rate of the eluate was very slow, even with suction.

Using the buffer pH 11 (K_2CO_3 solution) resulted in more interferences compared with water solution pH at 11. However, these impurity peaks did not interfere with the

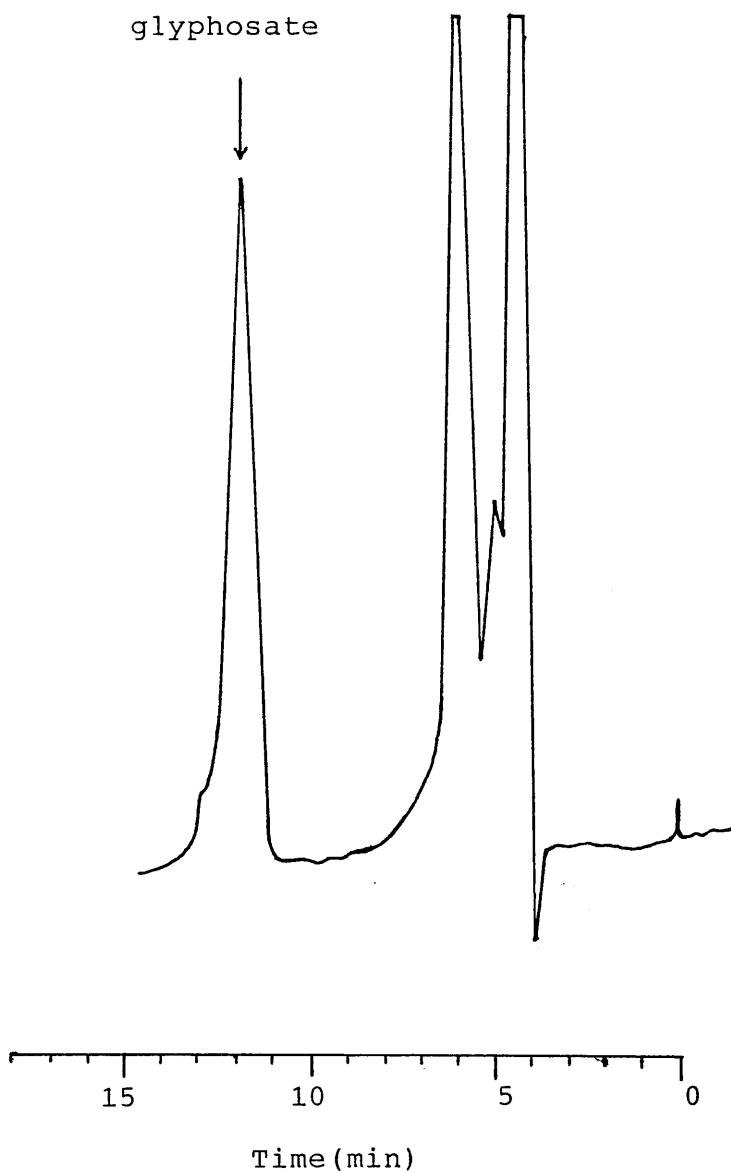


Figure 2.3 Chromatogram of 1ng (10µl of 0.1ppm) glyphosate standard after derivatization.

Conditions :

Packing material : APS hypersil.

Column : 25cm x 4mm.

Mobile phase : 25% acetonitrile/water
phosphate buffer pH=4.0 .

Flow rate : 1.0ml/min .

Detector : fluorescence. Excitation at 270nm.
Emission at 315nm.

Temperature : room temperature.

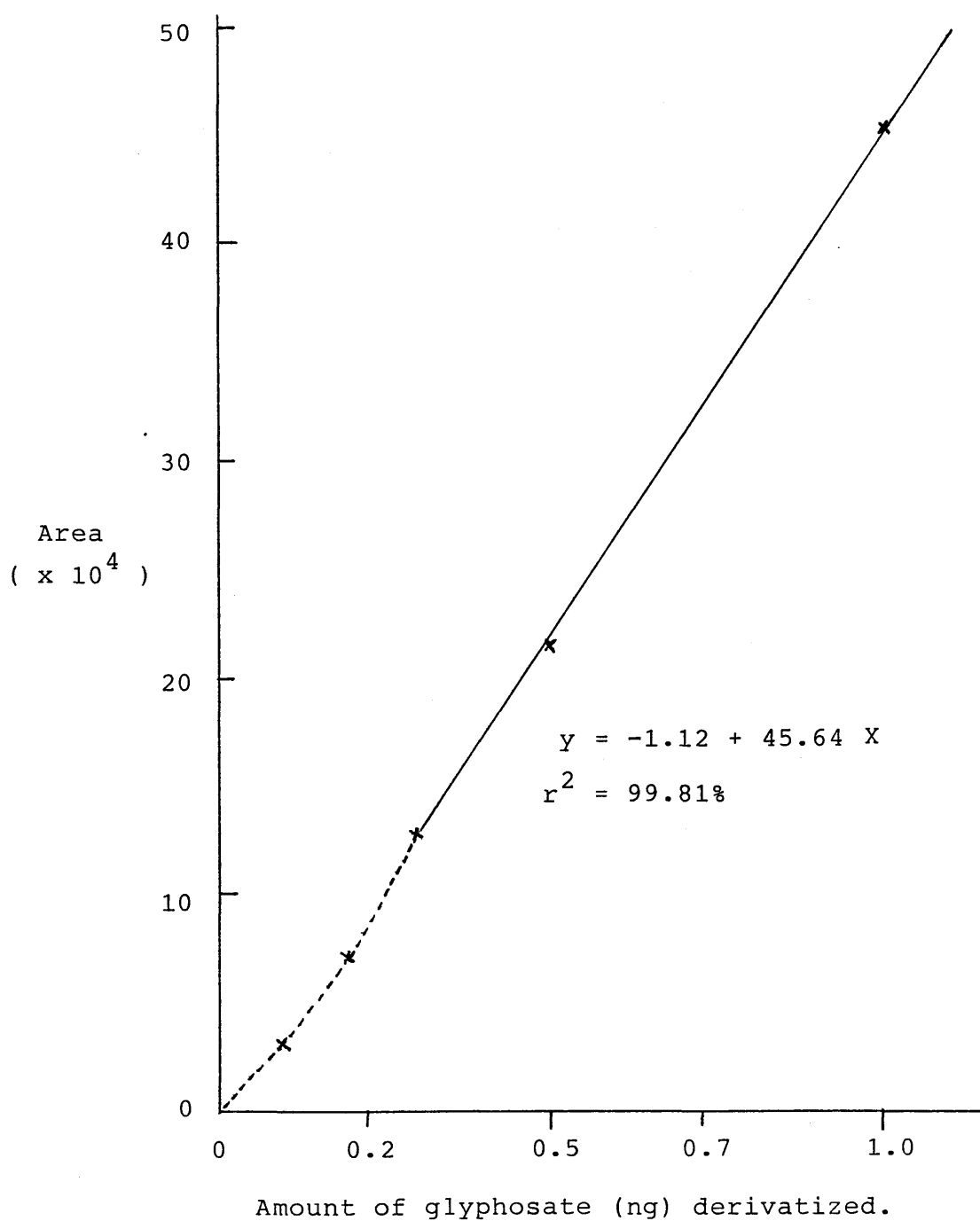


Figure 2.4 Graph of area against amount of glyphosate at low level (≤ 1 ng), after derivatization.

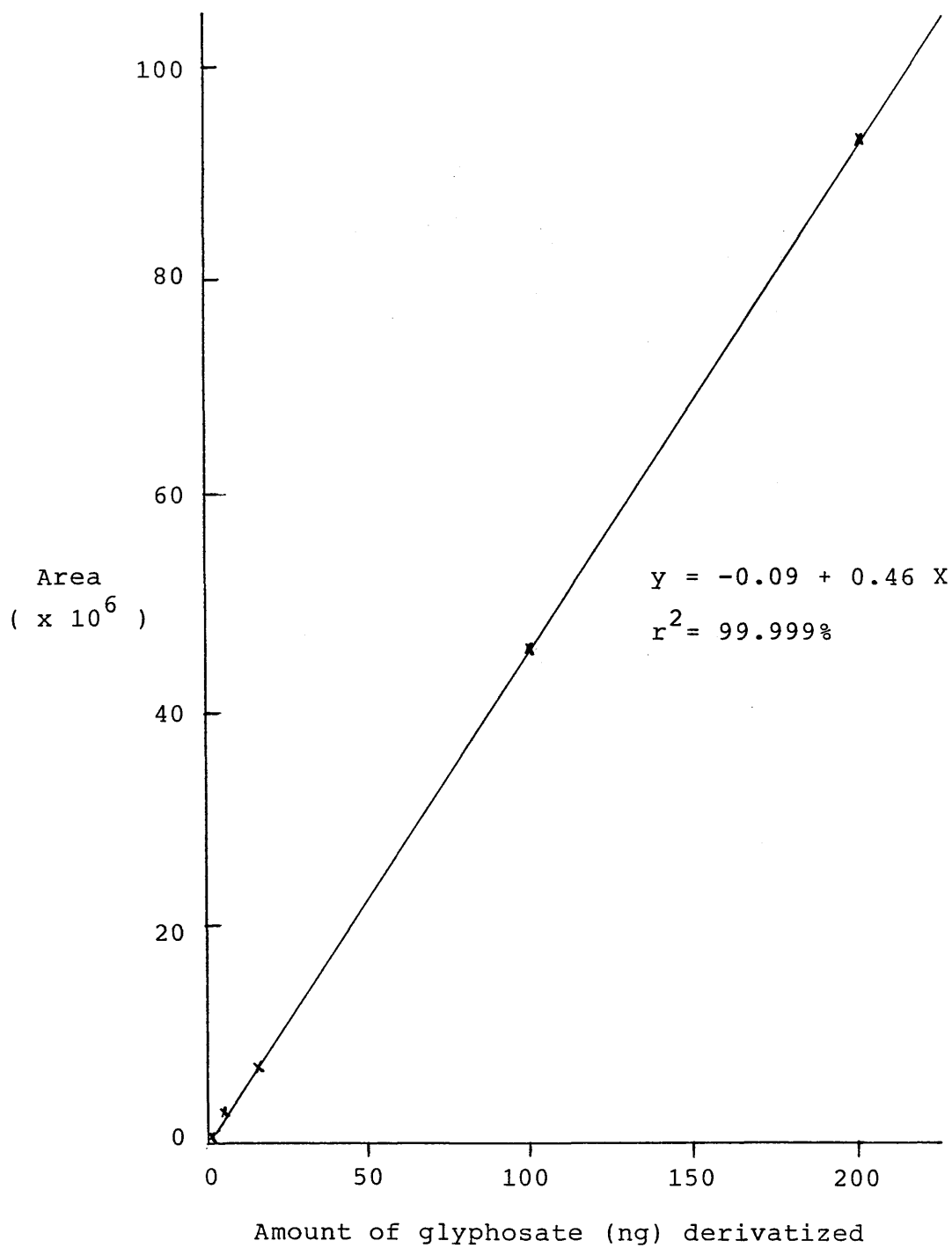


Figure 2.5 Graph of area against amount of glyphosate at high level (> 1 ng), after derivatization.

glyphosate peak although they did increase analysis time by a few minutes.

The above results also showed that an activated carbon column at pH 11 could adsorb a certain amount of glyphosate. This could be seen clearly when 0 % recovery for 0.1 ug of compound was obtained compared with a reasonably high recovery at the higher concentration of 10 ug of compound. Higher recovery could be obtained especially at lower levels of sample by using more elution volume but this alternative was excluded because it also resulted in higher interference from the impurities.

The sensitivity of the method of detection could be seen in figure 2.3. Linearity for glyphosate standard was 0.3 - 200 ng (figures 2.4 and 2.5). Higher than 200 ng was not checked because at this level it would give too high glyphosate residues in crops to be likely. However it was reported that linearity was obtained from 1 - 2000 ng by using the same HPLC column but with a different mobile phase (Roseboom and Berkhoff, 1982). Chromatograms of the potato sample extract before and after the activated carbon clean-up are given in figures 2.6 and 2.7. It was clear that without clean-up, the determination of glyphosate especially at low level was impossible due to interference of co-extractives.

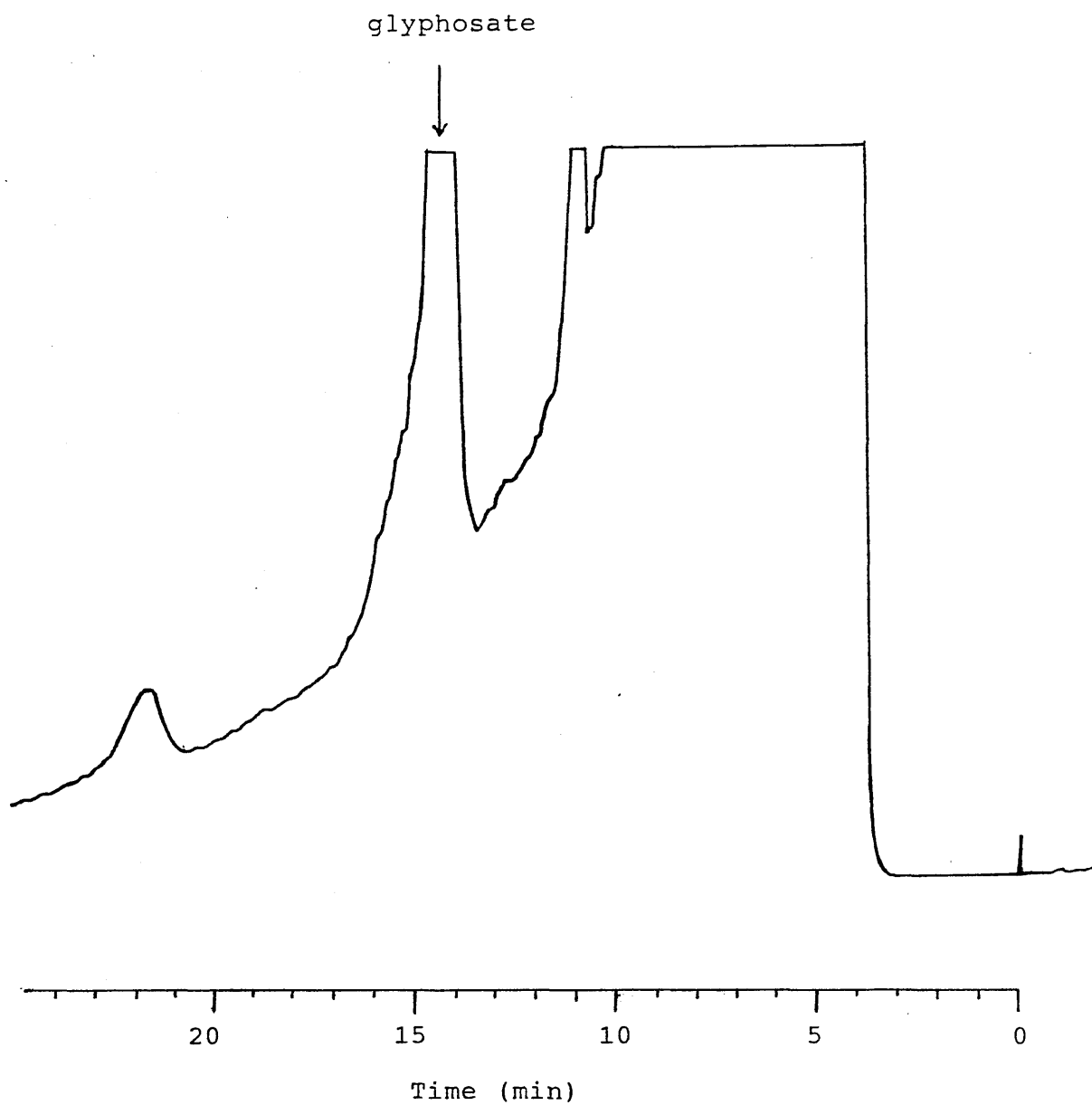


Figure 2.6 Chromatogram of 10ppm glyphosate spiked potato extract before activated carbon clean-up.

Conditions : as for figure 2.3 .

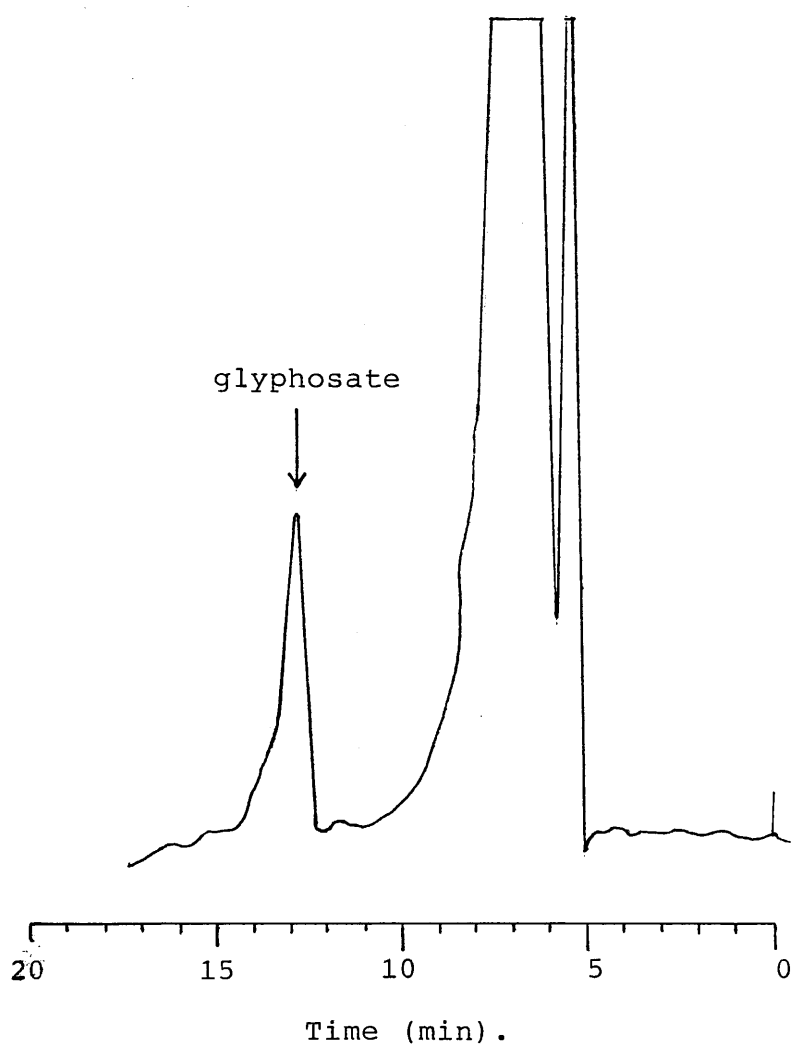


Figure 2.7 Chromatogram of 10ppm glyphosate spiked potato extract after activated carbon clean-up.

Conditions : as for figure 2.3 .

2.5.4.4 Use of Norit Activated Carbon.

The results in table 2.12 showed that a mixture of powdered activated carbon (Ultrisorb) and semi coarse (Chemviron) with ratio 1 : 2 could be used as a clean-up material for the determination of glyphosate residues in potatoes. However these types of activated carbons were no longer easily available commercially hence their effectiveness as clean-up materials was not investigated any further at this stage.

Other brands of activated carbon needed to be evaluated so that a choice could be made for future analysis. Activated carbons from Norit company were chosen because they are available in many forms from powder to granular compared to other products which are normally available in powder form only. For this purpose, Norit SX 1G (powder form) and Norit Azo whose particle size is coarser than Norit SX 1G but finer than the semi coarse of Chemviron evaluated earlier, were used. Particle size range of Norit SX 1G is 70-75 % above 10 micron and for Norit Azo is 99 % above 44 micron.

The method used throughout the experiment was as mentioned earlier in evaluating Ultrisorb and Chemviron activated carbons.

Based on previous observations, the ratio of 1:3 and 1:2 of Norit SX 1G to Norit Azo were evaluated. Flow rate was controlled by suction.

2.5.4.5 Results and discussion.

It was found that the ratio 1:2 gave a better recovery than 1:3 as had been obtained from the mixture of Ultrasorb and semi coarse (Chemviron) activated carbons. From this result and previous findings, the ratio 1:2 of Norit SX 1G to Norit Azo was used throughout the subsequent experiments.

Various elution volume fractions were collected. Recovery and influence of impurities in these fractions were determined. To reduce the effect of adsorption of the compound by the column, i.e. to increase the recovery, more sample volume was passed through the column especially at low levels of glyphosate. The improvement brought about by this modification could be seen in table 2.13.

Table 2.13 Effect of using more sample and collecting more elution volume on recovery.

Sample concentration	sample volume used (ml)	elution volume (ml) (total)	recovery %
10 ppm	1	13	53
	2	15	92
5 ppm	1	13	43
	2	14	50
	2	15	72
	3	15	88
1 ppm	1	13	39
	2	15	72
	3	15	79
0.5 ppm	2	13	33
	2	14	66
	2	15	68
	3	15	72
0.1 ppm	4	13	32
	4	15	53
	5	15	57*

* peak shape was distorted by the impurities and quantitation was difficult.

From comparison of table 2.12 and 2.13, it was found that the Norit activated carbons were slightly less effective at retaining the impurities and passing through the compound from potato extracts as compared to the mixture of Ultrasorb and Chemviron semi coarse ones used earlier. The recovery by Norit carbon was 92 % using 2 ml of 10 ppm sample and 98.1 % recovery was obtained with mixture of Ultrasorb and Chemviron semi coarse activated carbons using less sample volume i.e. 1 ml of the same concentration.

One of the reasons why Norit carbons were less effective was because the particle size of Norit Azo was smaller than Chemviron semi coarse. Differences in particle size meant that the mixture of Norit SX 1G and Norit Azo could adsorb material quite quickly compared to a mixture of Ultrasorb and Chemviron semi coarse. This was due to its higher surface area and the greater elution volume needed to desorb the glyphosate. Simultaneously, desorption of impurities also occurred as could be seen by the chromatograms of the fractions of elution volume where the effect of impurities increased in order 15 > 14 > 13 ml fraction in spite of the higher recovery obtained from 15 ml elution volume fraction. Elution volumes of more than 15 ml could not be evaluated for recovery because the impurities present gave very intense peaks.

Although Ultrasorb and Chemviron activated carbons performed better, their disadvantage was that they were no longer commercially available. Norit activated carbons could still produce a reasonably good recovery for the

determination of glyphosate residues in potatoes and had the advantage of being easily available commercially.

Recovery could be improved by using more sample volume passed through the column where the adsorption effect would be reduced especially at low level (table 2.13). As had been expected when more sample volume was introduced to the column, more impurities would affect the determination. This could be seen in table 2.13 when higher recoveries could be gained by introducing 5 ml of 0.1 ppm sample instead of 4 ml but the peak shape was distorted by closely eluting co-extractives which made it quite difficult for quantitation (figures 2.9 and 2.10). Figure 2.8 shows a chromatogram of a potato extract without glyphosate standard addition for comparison with figures 2.9 and 2.10. From these results it could be concluded that the volume of the sample that should be passed through the column would be 4 ml or less, depending on the amount or concentration of glyphosate in the potato.

Minimum detection limit by this method was 0.05 mg/kg (see figure 2.11).

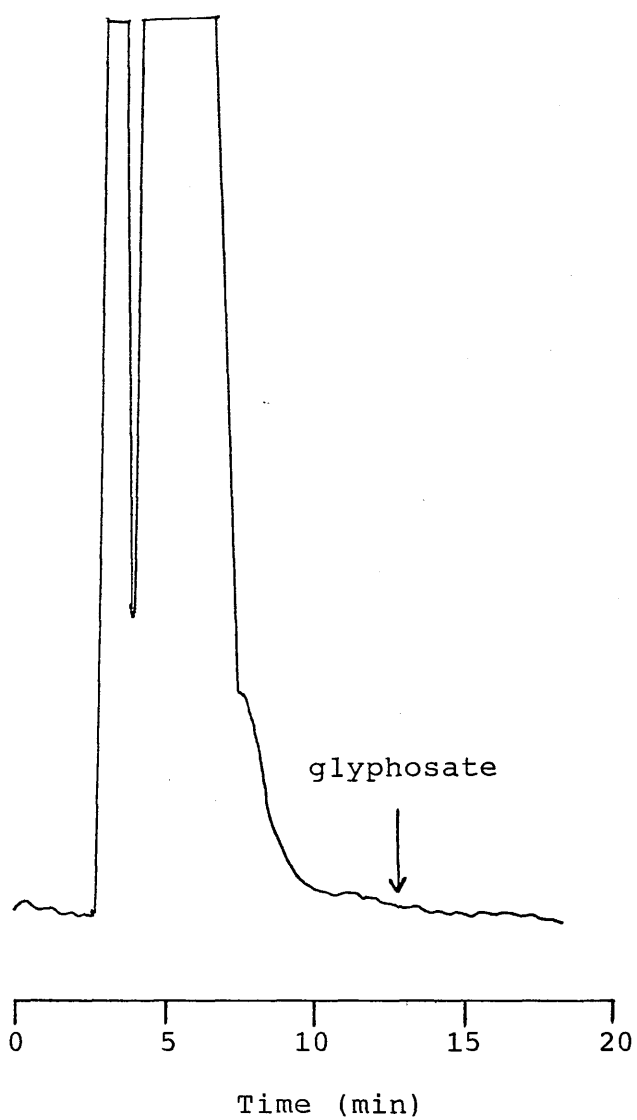


Figure 2.8 Chromatogram of blank potato extract.

Conditions : as for figure 2.3 .

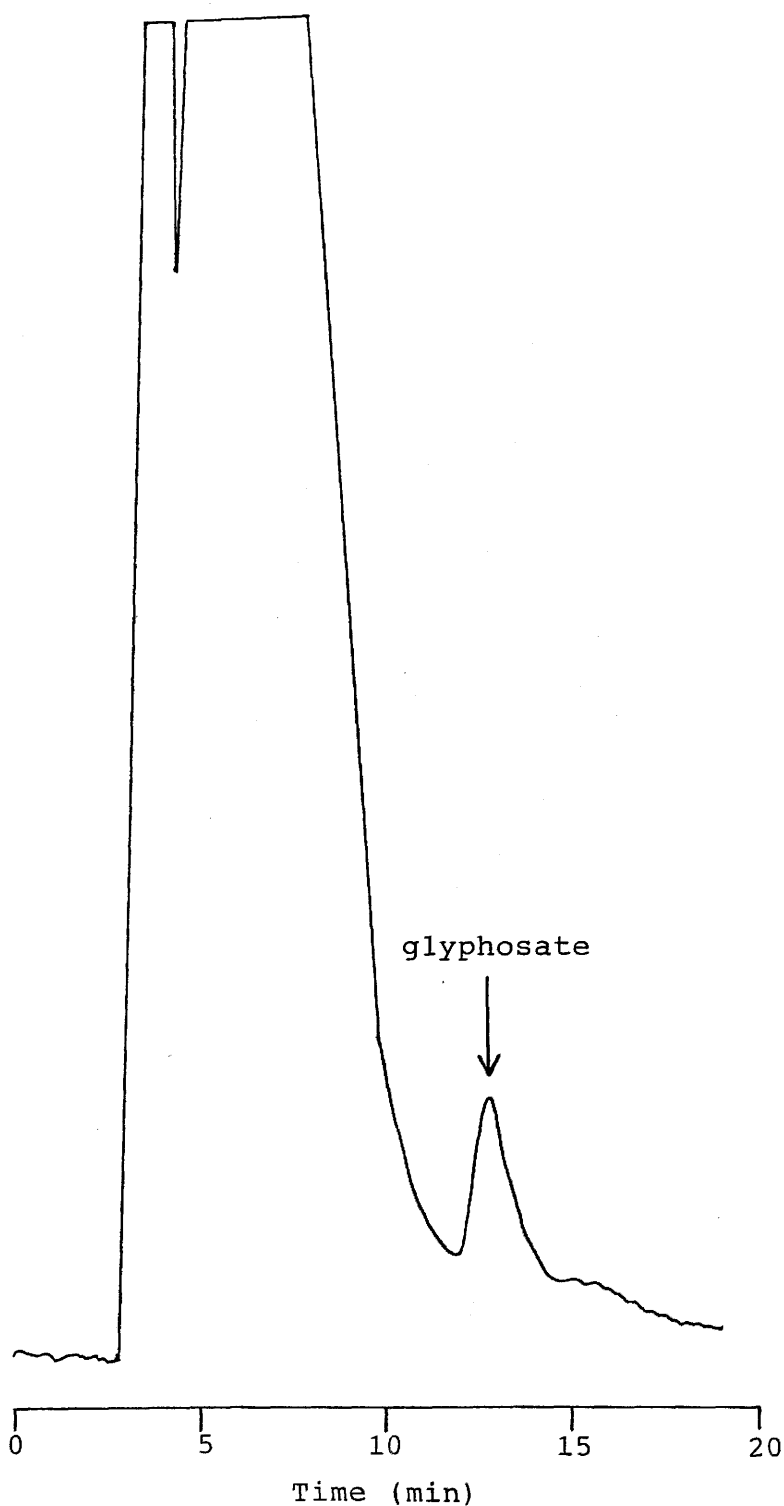


Figure 2.9 Chromatogram using 4ml 0.1ppm glyphosate spiked potato extract introduced onto clean-up column.

Conditions : as for figure 2.3 .

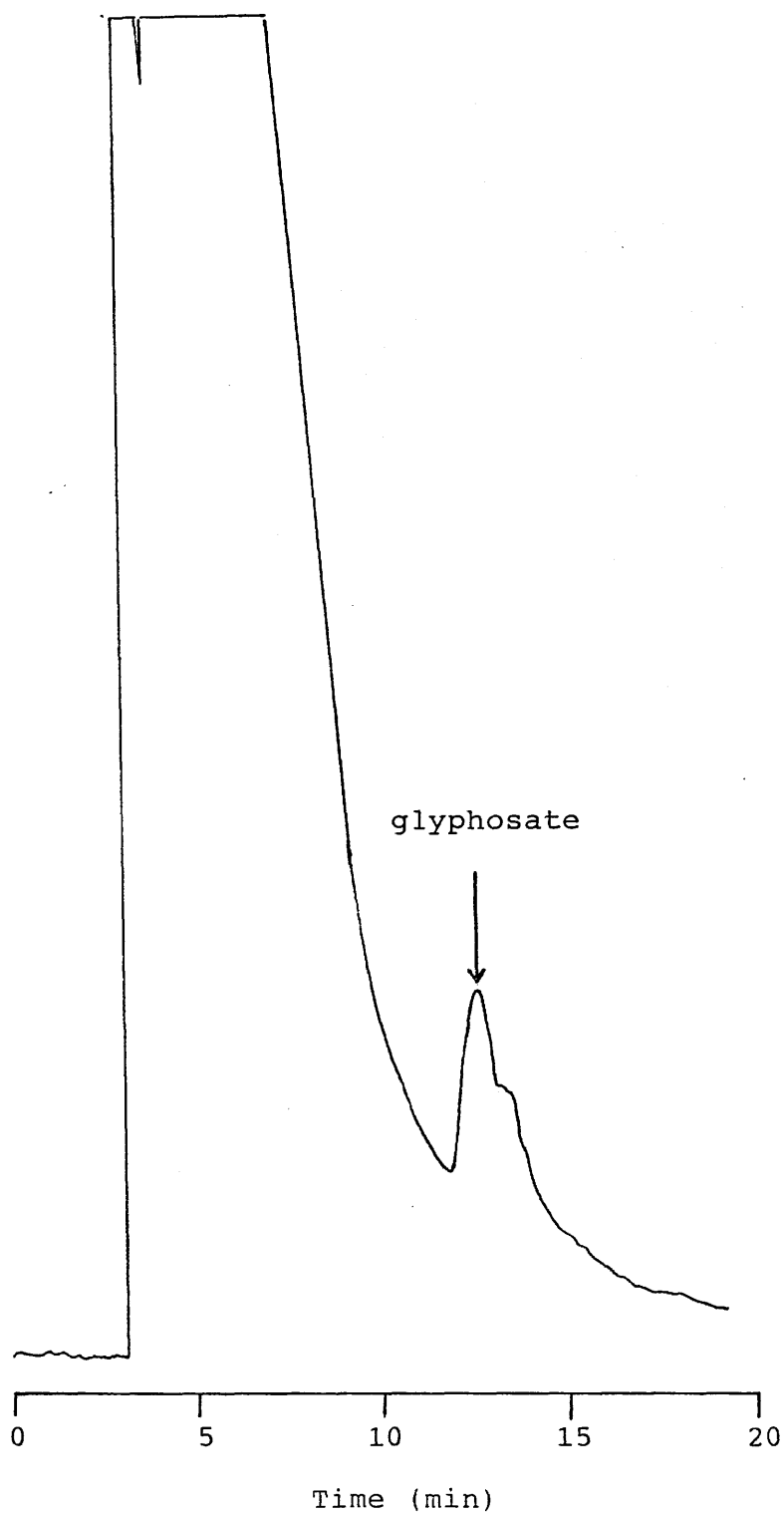


Figure 2.10 Chromatogram using 5ml 0.1ppm glyphosate spiked potato extract introduced onto clean-up column. Compound peak distorted by the impurities.

Conditions : as for figure 2.3 .

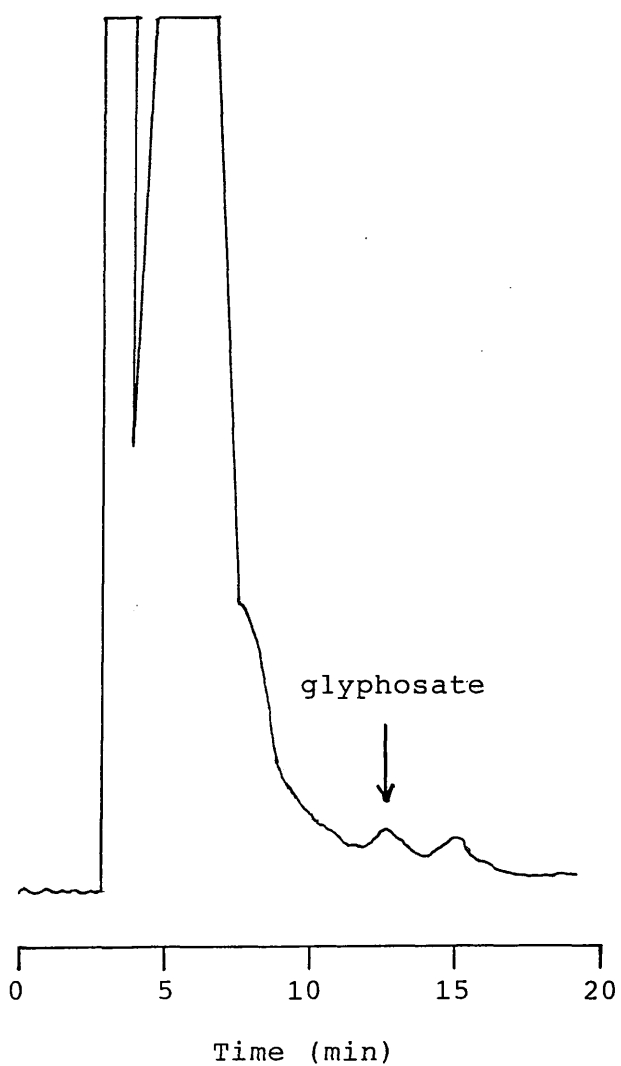


Figure 2.11 Chromatogram of 0.05ppm glyphosate spiked potato extract.

Conditions : as for figure 2.3 .

2.6 RECOMMENDED METHOD FOR ANALYSIS OF GLYPHOSATE RESIDUES IN POTATOES.

From the above experiments and results the recommended method for analysis of glyphosate residues in potato tubers is as follows :

Procedure : 25 g chopped potato, 25 ml chloroform and 50 ml water were placed in 500 ml stainless steel blender jar. The mixture was blended at medium speed for 10 minutes. The jar was rinsed with 2 x 20 ml water and the content and rinses were placed in 250 ml centrifuge bottle. After centrifugation at 10,000 rpm for 20 minutes, the supernatant was filtered through Whatman no.1 filter paper into 500 ml round bottom flask followed by 2 x 20 ml water used for rinsing the centrifuge bottle. Rotary evaporation was employed at a temperature below 40 °C to reduce the volume to about 5 ml.

The extract was adjusted to pH 11 firstly with 10 M KOH and finally with 1 M KOH and the final volume was made to 25 ml. It was then filtered through Whatman no.1 filter paper and the extract was stored in the fridge until analysed.

Activated carbons (50 g of Norit SX 1G and 50 g of Norit Azo) were each pre-treated by soaking for about 2 hours in concentrated HCl. The excess HCl was removed by washing with deionized water and then with slightly alkaline deionized water until neutral. Then the carbons

were dried in the oven (110 °C), cooled and stored in tightly sealed containers.

A glass column (1 x 40 cm) equipped with a glass stopcock was plugged with glass wool. 5 g activated carbon (mixture of Norit SX 1G and Norit Azo at ratio 1:2) was added to the column. The column then was equilibrated with K_2CO_3 (10 g/l). 4 ml or less of the sample extract was added to the top of the column and a 30 ml bottle was placed under the column as a receiving vessel. Glyphosate was eluted from the column with the K_2CO_3 solution. The first 15 ml of eluate was collected and retained. The flow rate was increased by water suction.

Derivatization : 4 ml of the collected eluate was placed together with 2 ml acetone and 2 ml 0.1 M FMOCCl in acetone, into a teflon capped 30 ml bottle. The solutions were incubated at 23 °C for 20 minutes. Three separate 5 ml portions of diethyl ether were used to remove excess reagents. The aqueous solution was placed on a hot water bath for a few minutes to remove the residual ether and after cooling to room temperature, the volume was adjusted to 5 ml using a volumetric flask. From this solution 10 ul or less were injected in the HPLC column (the solution was filtered through 0.45 um filter paper before injection onto the HPLC).

HPLC : The HPLC system consisted of a Waters Associates model 6000 A pump, a Rheodyne model sample injection valve equipped with 20 ul sample loop and Shimadzu Fluorescence HPLC detector. Chromatograms were recorded on a Spectra Physic SP 4290 integrator. Excitation was at 270 nm and emission at 315 nm. Isocratic operation was conducted at 1.0 ml/min with pH 4 phosphate buffer (0.1 M) containing 25 % acetonitrile by volume. Separations were achieved on an APS hypersil column (25 cm x 4 mm).

Column comparisons were made with glyphosate standards which were similarly derivatized.

Note : Any comparable instruments would be appropriate for carrying out this determination.

CHAPTER 3

EFFECT OF GLYPHOSATE ON FOOD TUBERS (POTATO).

3.1 INTRODUCTION.

Applications of glyphosate are recommended before cereals are harvested and at the same time there is a potential for damage from drift. Drift damage occasionally happens to horticultural crops near to cereal crops treated pre-harvest with glyphosate. It has been reported that a number of horticultural crops have been severely damaged by drift of glyphosate applied around glass houses or between rows of bushes (Elliot and Wilson, 1978).

Glyphosate can cause more drift damage than 2,4-D oil-soluble amine, MSMA, aminotriazole, cacodylic acid, paraquat or dalapon (Lange and Schlesselman, 1975). Accidental damage can occur either by drift during weed control operations within a crop or by drift from one field to another.

Consideration of its ease of translocation and the long list of reported actual or potential instances of damage due to glyphosate in a range of species e.g. cane fruits (Davidson, 1975), cotton (Anon, 1973; Kleifeld, 1976; Tollervey et al., 1980), maize (Copper, 1975) plantain (Liu et al., 1981), *Prunus* spp (Rom and Talbert, 1973), sugarcane (Ching et al., 1976), tanier (Liu and Acevedo-Borrero, 1980), tea (Magambo and Kilavuka, 1982),

tomato (Romanowski,1974), valencia orange (Toth and Morrison,1977) and vine (Barralis et al.,1973; Kafadarof and Poisson,1973), suggest that damage due to drift is likely to become more common and glyphosate gives more severe effects than other herbicides.

Losses of small but significant amounts of chlorophenoxy herbicides from fields during and following treatment have been recognised as a major problem for many years (Yates et al.,1978; Elliot and Wilson,1983). Small size spray drops may be transported from treated areas by light and variable winds, particularly under temperature inversion (warm air overhead) conditions which confine movement close to the ground. Widespread, low level damage symptoms in sensitive plants have occurred several miles from the application site (Yates et al.,1978). Due to its properties, glyphosate does not cause the damage associated with vapour transfer compared with some other compounds (Wiese,1976; Shaw and Bruzzere,1979).

Glyphosate is phloem mobile, so application of this compound to plant parts that are naturally 'exporting photo-assimilates' leads to greater translocation compared with other parts of the plants (Sprankle et al.,1975; Wyrill and Burnside,1976; Coupland and Caseley,1979; Gougler and Geiger,1981; Smid and Hiller,1981). As a tuber or rhizome crop, the potato tuber will receive more glyphosate than any other parts of the plant when glyphosate is applied to the leaf area. This could happen when glyphosate is used to kill weeds adjacent to potato fields but drift, caused by wind, affects the potato area.

This is particularly important in Scotland where a large percentage of potato crops is grown for seed and therefore any chemical which could affect germination is an obvious cause for concern. Spraying chemicals in Scotland where it can be quite windy coupled with the fact that cereals and seed potatoes are often grown in adjacent fields means that contamination of potatoes by drift is a distinct possibility. This laboratory has received samples of seed tubers which although healthy looking, failed to grow. Disease and contamination by sprout suppressant chemicals were ruled out as reasons for the failure to grow. Glyphosate was thought to be a potential cause but the difficulty of analysis meant that this could not be proved.

From the above information the purposes of the experiments were as below :

- i. To simulate wind drift damage of glyphosate in potato tubers by spraying it on the potato plants at different concentrations near the time of harvesting.
- ii. To study especially the physical effect of these glyphosate treatments on potato tubers under normal storage conditions.
- iii. To determine residue levels of glyphosate in these glyphosate treated tubers.
- iv. To correlate the relationship between the amount of glyphosate applied, glyphosate content in tubers and the effects on tubers under normal storage conditions.

- v. To predict the effect on potato tubers when glyphosate accidentally is sprayed on potato plants or drifts to adjacent potato fields while applied to kill weeds.
- vi. To study the field growth performance of glyphosate treated tubers.

The potato was chosen for the above study because it is particularly well suited for the following reasons :

- a. It is a commonly consumed food material.
- b. The tuber does not give as fast response as the leaf does, when chemical is applied to the plant.
- c. The main barrier to chemical translocation in plant is leaf cuticle. For potato leaf this cuticle is not a great hindrance because it is relatively thin and delicate (<0.2 mg/cm) with only small deposits of surface wax (<0.05 mg/cm) (Martin and Juniper, 1970). So this compound could quite easily penetrate the leaf and translocate to other parts of the plants.

3.2 FIELD AND STORAGE EXPERIMENT.

3.2.1 Site details and plot layout.

The site chosen for the field experiment on the effect of glyphosate on potato tubers was a small portion of a potato commercial farm, Arkleston Renfrew Farm, Scotland (Grid Reference NS 508655).

Sixteen treatment plots were employed so that each treatment was replicated four times in a randomised complete block design. Plot layout is shown in figure 3.1. Total area for this field experiment was ca. 72 meter square; it consisted of two 3m drills 75 cm apart. Each drill contained 15 tubers, 20 cm apart of cv. Pentland Squire. There was no separation or crop boundary amongst the blocks and treatments. Seed tuber treatments and agricultural practices were based on commercial treatments.

3.2.2 Application of glyphosate.

For this experiment, glyphosate in commercial formulation Round-up (Monsanto,U.K) was applied to the treatment plots by hand held sprayer (5 litre capacity) 10 days before harvesting. Spraying was done on 1 September 1986. Four treatment levels were applied corresponding to :

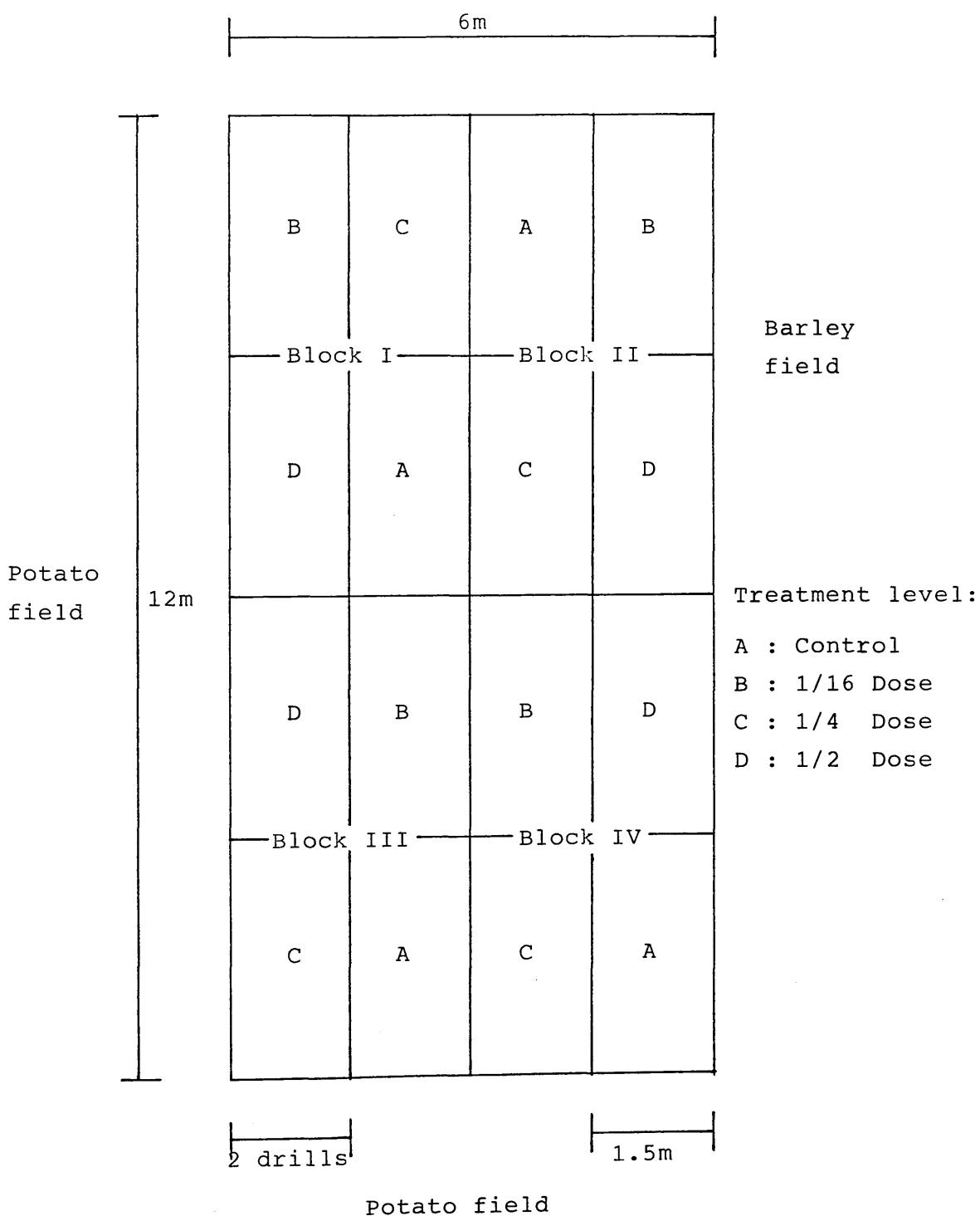


Figure 3.1 Plot layout of the field experiment.

- a. Treatment A - deionized water as control.
- b. Treatment B - Round-up at 1/16 of normal recommended application rate.
- c. Treatment C - Round-up at 1/4 of normal recommended application rate.
- d. Treatment D - Round-up at 1/2 of normal recommended application rate.

Normal application rate as recommended by the manufacturer was 4 litres in 250 litres water per hectare which was equivalent to 1.44 kg.a.i./ha.

For the above experiment, commercial formulation Round-up was diluted with deionized water. Care was taken during each application so that plot canopy was evenly covered by each spray treatment. During spray application adjacent plots were covered with polyurethane plastic sheets to minimise the drift effect between plots.

3.2.3 Tuber storage.

In order to monitor the effect of glyphosate on the tuber under normal storage conditions, the plants were harvested 10 days after applying the glyphosate, i.e. 11 September 1986. Then they were left at room temperature for about a week for wound healing to take place. From each plot, 10 kg of tubers were taken and put in a cardboard box. They were stored in a temperature controlled room at 9 °C with relative humidity more than 90 %. These storage potatoes were checked for sprouting,

number of eyes open and their resistance to disease or abnormality from time to time.

3.2.4 Residue analysis.

For determination of glyphosate residue level in these tubers, 6 healthy tubers were chosen from each plot (box). Then they were washed with deionized water and kept in the deep freeze until required for analysis.

For the residue analysis, 6 tubers were quarterly sampled and chopped to a homogenous sample, and 25 g was taken for analysis. Method of analysis was as mentioned in chapter 2.

3.2.5 Effect of glyphosate on tuber sprouting, number of eyes open and disease/rotting.

For sprout length measurements, 25 tubers were randomly chosen from each plot(box), and the longest sprout length from each tuber was recorded. At the same time, the number of eyes open and disease effects were also recorded. A 0-4 scale was used to assess the disease effect, using the following ratings :

disease effect	rating
no disease	0
slight	1
medium	2
severe	3
very severe	4

For these purposes tubers were kept for 7 months under normal storage conditions.

After checking for normality, all the data taken were statistically analysed using two-way analysis of variance and were tested by F value at 5 % and 1 % levels (Dowdy and Wearden, 1983; Gomez and Gomez, 1984). If there was a significant difference, it was further checked by Duncan's new multiple range test for differences between overall treatment means at 5 % level.

3.2.6 Subsequent field performance of glyphosate treated tubers.

Growth performance of these glyphosate treated potato tubers were evaluated in natural conditions in the field. After 7 months of storage, 10-13 healthy tubers were randomly chosen from each plot/box and then they were grouped together according to their treatments (control, 1/16 dose, 1/4 dose and 1/2 dose). From each treatment, 25 tubers were randomly chosen and planted on 29 April 1987 in two 10 m drills 20 cm apart. Their growth performance was checked from time to time without applying any fertilizer or other agricultural practices. Plants were harvested on the 3 November 1987. Plot lay out of this experiment is shown in figure 3.7.

If the plants looked abnormal i.e. stunted and or showed signs of chlorosis, they were assessed as unhealthy.

3.3 RESULTS AND DISCUSSION.

The amount of glyphosate received by each plot from each treatment is as detailed in table 3.1.

Table 3.1 Estimated glyphosate loading of each treatment.

Treatment code	A	B	C	D
Treatment level	control	1/16 dose	1/4 dose	1/2 dose
Plot numbers	4,5,12,16	1,6,10,13	2,7,11,15	3,8,9,14
Quantity applied to each plot (g/4.5 m ²)	0	0.04	0.16	0.32
Quantity applied per unit area (kg/ha).	0	0.09	0.36	0.72

Most of the tubers produced from this experiment were ware grade (> 57 mm), only a small amount in the form of seed grade (32-57 mm).

Cv. Pentland Squire has a quite long dormancy period under cold conditions. It showed sprouting after about 4 months of storage.

Glyphosate injury symptoms to the potato plants were not observed. Smid and Hiller (1981) found that the first visible symptom of glyphosate injury was a slight epinasty of potato foliage 1 day after treatment. Two days after the herbicide application, the small leaves at

the growing points became chlorotic. Chlorosis gradually extended down to the more mature foliage and 7 days after treatment at least 50 % of the foliage was necrotic regardless of rate applied.

The rate of application for this experiment had been limited to a maximum of 0.72 kg.a.i/ha (1/2 dose) for the following reasons :

- i. 1/2 dose of ordinary rate of application is normally higher than the plant would receive from drift effect.
- ii. At rate 0.56 kg/ha an increased number of daughter tubers were produced. Slightly less daughter tubers formed at rates greater than 0.56 kg/ha. Plants receiving 1.12 or 2.24 kg/ha produced daughter tubers either partially or completely decomposed (Smid and Hiller,1981).
- iii. No significant reduction of dry matter from potato plants which received more than 1.12 kg/ha was noted (Smid and Hiller,1981).

When glyphosate was applied to potato plants before tuber initiation, it moved basipetally into the roots and acropetally into the apical meristem of the foliage as a typical sink (Smid and Hiller,1981). The same has been observed in several other perennial weed species (Claus and Behrens,1976; Gottrup et al.,1976; Sprankle et al.,1975; Wyrill and Burnside,1976; Zandstra and Nishimoto,1977). As the plants mature new root growth decreases and the assimilate flow shifts to the new

rhizomes and tubers. Newly developing daughter tubers may accumulate increasing quantities of glyphosate.

3.3.1 Sprout length.

The results are presented in table 3.2.

Two way analysis of variance showed that the treatments had a highly significant effect while the blocks had no significant effect on the sprouting of the tubers over a storage period.

Different treatments gave different effects on sprouting of tubers. This observation was expected for glyphosate as it has been shown to closely follow the same distribution pattern within plants as the photo-assimilates (Gongler and Geiger, 1981; Dewey, 1982). In mature potato plants it was expected that glyphosate would be transported to the tubers as the main sink .

There were variations in sprout length among the blocks which received the same treatment although statistically they were not significantly different. The variation could be explained by the fact that if the plants had initiated more tubers, i.e. more 'sink', the herbicide would be diluted within the plants compared to plants with less tubers. Concentration of glyphosate in high tuber bearing plants would be less than low tuber bearing plants, so their rates of survival should be higher (Lolas and Coble, 1980).

Table 3.2 (A). Potato tuber sprout length (mm) grouped by treatment and block.

Treatment	mean sprout length (mm) ^a				Treatment	s.d. ^b

	Block				mean	
	I	II	III	IV		
A - control	202	92.2	101	210	151.3	63
B - 1/16 dose	78.3	131	74.0	95.2	94.7	26
C - 1/4 dose	11.9	22.4	13.2	41.9	22.3	14
D - 1/2 dose	8.68	10.7	5.68	38.2	15.8	15

a - average of 25 tubers

b - standard deviation

(B). Analysis of Variance

Source of		Sum of	Mean sum	Observed	Tabulated F		
variation	df	squares	of squares	F	p 0.05	0.01	

Treatments	3	49774	16591	12.58 **	3.86	6.99	
Blocks	3	3470	1157	0.88 ns			
Error	9	11868	1319				
Total	15	65112					

** = highly significant difference (p<0.01)

ns = not significant

However Lovell and Booth (1967) and Moorby (1968, 1970) found that there was no relationship between the amount of photo-assimilate compounds transported to the tubers and their fresh weight.

Lutman (1979) found that despite the high metabolic activity of developing tubers and thus their high sink capacity, not all the tubers formed from the glyphosate treated stems were showing glyphosate damage for the following reasons :

- i. Lack of symptoms on the tubers does not necessarily mean they were unaffected; previous experiments showed that apparently undamaged tubers sometimes failed to form healthy plants (Lutman and Richardson, 1978).
- ii. Tubers do not grow at the same rate through their life (Moorby, 1968; Gray, 1973). So slow growing ones may absorb less glyphosate than a faster growing one.
- iii. Detailed ^{14}C studies by Gray and Smith (1973) showed that a high percentage of assimilates applied to one leaf was translocated to tubers arising from that portion of the stem within an arc of 45° from the supply leaf. Hence some tubers may receive more herbicide from specific leaves than others.

From above observations one could expect the variation in sprout length within the same treatment as was noted here. From two way analysis of variance, there was

a highly significant effect of the treatments.
 Significant differences between the means for different treatments were determined using Duncan's new multiple range test.
 Significant difference (S.D) was determined by the formula :

$$S.D = d_{\alpha,r,a(n-1)} \sqrt{\frac{MS_e}{n}}$$

where

- d = critical value for Duncan's new multiple range test at α level.
- r = span r ranked sample averages
- MS_e = mean sum of squares for error.
- n = treatment group
- a(n-1) = degree of freedom for error

where :

$d_{\alpha,2,a(n-1)} \sqrt{\frac{MS_e}{n}}$	Apply to bottom diagonal
$d_{\alpha,3,a(n-1)} \sqrt{\frac{MS_e}{n}}$	Apply to second lowest diagonal
.	.
.	.
.	.
$d_{\alpha,a,a(n-1)} \sqrt{\frac{MS_e}{n}}$	Apply to top diagonal

To reject null hypothesis (H_0): $u_i = u_j$ when y_i and y_j span r ranked sample averages, it is necessary that

$$Y_i - Y_j \geq d_{\alpha, r, a(n-1)} \sqrt{\frac{MS_e}{n}}$$

It was calculated that the significant difference at various span r ranked sample averages as follow :

r	value (mm)
2	58.09
3	60.64
4	62.11

Significant differences between the treatment means are presented in table 3.3.

Table 3.3 Differences between the treatment means of sprout length of potato tubers, following glyphosate treatment.

Significant difference	No significant difference
A and C	A and B
A and D	C and D
B and C	

There was no significant reduction of sprout length between the rate of glyphosate application at 0.09 kg/ha and controls. Increasing the rate from 0.36 kg/ha to 0.72 kg/ha also did not produce a significant reduction of sprout length.

Attempts were also made to examine the relationship between the mean sprout length and treatment levels. A linear regression equation was not suitable for this relationship; a curve regression equation would be more appropriate. The graph of mean sprout length against treatment rate is given in figure 3.2 with the equation as below :

$$S = 120 e^{-3.19 T}$$

where

S = sprout length (mm)

T = treatment rate (kg/ha).

r^2 (coefficient of determination) was 88 %.

From figure 3.2 it was found that the sprout length decreased rapidly at low level but this decrease slowed with higher levels of treatment. Other transformations such as log, square root and minus reciprocal were also tried but none of them gave a better relationship.

For relating amount of glyphosate load to potato plants due to drift effect, using sprout length as an indicator, the relationship as in figure 3.2 could be used to give a guide.

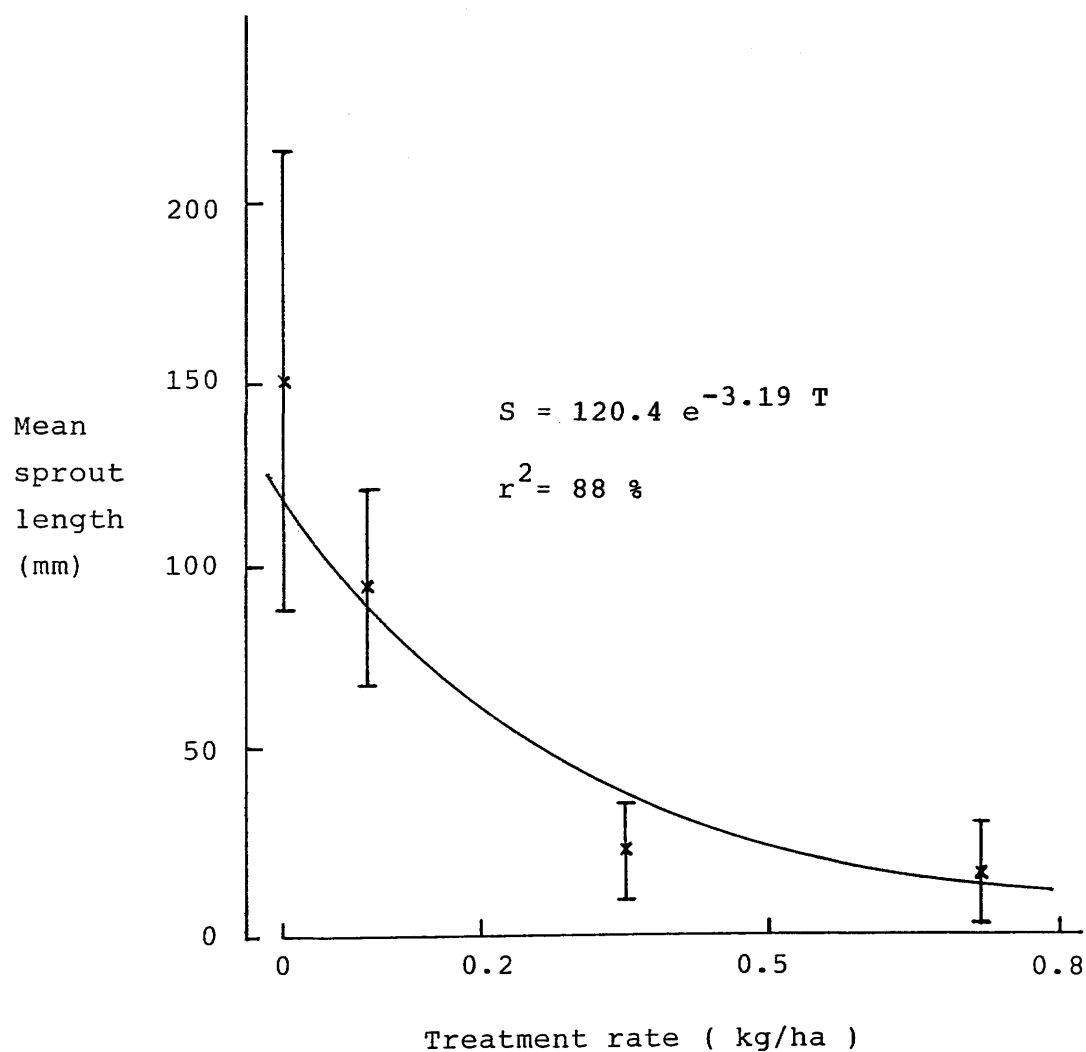



Figure 3.2 Graph of sprout length against treatment rate.

 - mean with associated \pm s.d .

3.3.2 Number of eyes open.

It was found that treated tubers especially at higher rates produced clusters of suppressed sprouts. This observation was almost the same as obtained by Smid and Hiller (1981), where they found that abnormal sprouts were produced from treated tubers when they were allowed to grow. This showed that glyphosate moved from foliage down to all eyes of tubers. It was not determined whether glyphosate moved via symplast or apoplast into the tuber or whether it accumulated in the eyes. The above observation indicates that glyphosate induced loss of apical dominance as normally one dominant sprout emerged from a single eye of the potato tuber.

The results are presented in table 3.4.

Two way analysis of variance showed that the treatments have highly significant effect while the blocks have no significant effect on the number of eyes open of the potato tubers over a storage season.

From table 3.4 it was found that mean number of eyes open increased up to 0.36 kg/ha (1/4 dose), then decreased again at 0.72 kg/ha (1/2 dose). The reason for the reduction in the number of eyes open at the highest treatment level was due to the disease/rotting effect on the tubers by that treatment (see table 3.5 in section 3.3.3). When tubers were affected by the disease/rotting, the number of eyes open at affected areas could not be counted because they were diminished by the rotting. Consequently the numbers of eyes open would be less than it should be. This could be shown clearly with a

Table 3.4 (A). Potato tuber number of eyes open grouped
by treatment and block

Treatment	mean number of eyes open ^a				Treatment	s.d. ^b

	Block				mean	
	I	II	III	IV		
A - control	2.04	3.24	3.84	2.64	2.94	0.77
B - 1/16 dose	3.64	2.88	3.80	4.80	3.78	0.79
C - 1/4 dose	4.28	4.76	5.24	4.64	4.73	0.40
D - 1/2 dose	1.72	2.84	3.24	5.12	3.23	1.4

a - average of 25 tubers

b - standard deviation

(B) i. Analysis of variance

Source of variation	df	Sum of squares	Mean sum of squares	Observed F	Tabulated F	p	0.05	0.01
Treatments	3	7.449	2.483	4.02 *	3.86			6.99
Blocks	3	4.586	1.529	2.47 ns				
Error	9	5.560	0.628					
Total	15	17.595						

* = significantly difference ($p < 0.05$)

ns = not significant

ii. Analysis of variance without data from
treatment D (1/2 dose)

Source of variation	df	Sum of squares	Mean sum of squares	Observed F	p	0.05	0.01
Treatments	2	6.438	3.219	8.87 *		5.14	10.92
Blocks	3	1.588	0.529	1.46 ns		4.76	9.78
Error	6	2.176	0.363				
Total	11	10.202					

* = significantly difference ($p < 0.05$)

ns = not significant

replicate of 1/2 dose treatment that had less rotting effect, when the number of eyes open was the highest (see table 3.4 and disease effect for block iv). Overall, when the treatment levels were increased the number of eyes open would be increased.

Significant differences between treatments were calculated by using Duncan's new multiple range test as mentioned in section 3.3.1. Data from treatment 0.72 kg/ha (1/2 dose) was excluded in the test because it did not represent the true picture of number of eyes open as mentioned above.

It was calculated that the significant differences at various span r ranked sample averages as follow :

r	value
2	1.20
3	1.24

From that value, it was found that treatment A and C were significantly different while treatment A and B, B and C were not. Application of glyphosate at rate 0.09 kg/ha did not result in any significant difference in increasing number of eyes open compared with controls. Increasing the rate from 0.09 kg/ha to 0.36 kg/ha also did not give a significant increase in number of eyes open.

Every eye of the tuber acts as a potential growing point. Although there was no significant effect on number of eyes open between the blocks, there was variability between the blocks. This variability could be expected, as in section 3.3.1 and also due to the following factors:

- i. Large differences in number of eyes open per seed tuber were found even from the same cultivar and grade (Svensson, 1969).
- ii. Tubers from the different block of the same treatment normally were different in size and, as observed by Allen (1978), the number of eyes open per tuber increased with tuber size. This relationship was not linear.
- iii. Rotting effect varied slightly between the experimental blocks.

A better relationship was obtained when the number of eyes open was plotted against the square root of treatment rate rather than against treatment rate itself. The graph of number of eyes open against square root of treatment rate is in figure 3.3(a). r^2 for this graph was 11.6 %. When data from treatment D was excluded from the graph as it was not representative of the true picture of the number of eyes open, the regression equation became :

$$E = 2.92 + 2.98 \sqrt{T}$$

where

E = number of eyes open

T = treatment rate (kg/ha)

a better relationship was obtained (figure 3.3(b)) with r^2 value 99.9 %. Other transformations such as log and minus reciprocal were also tried but none of them gave a better relationship.

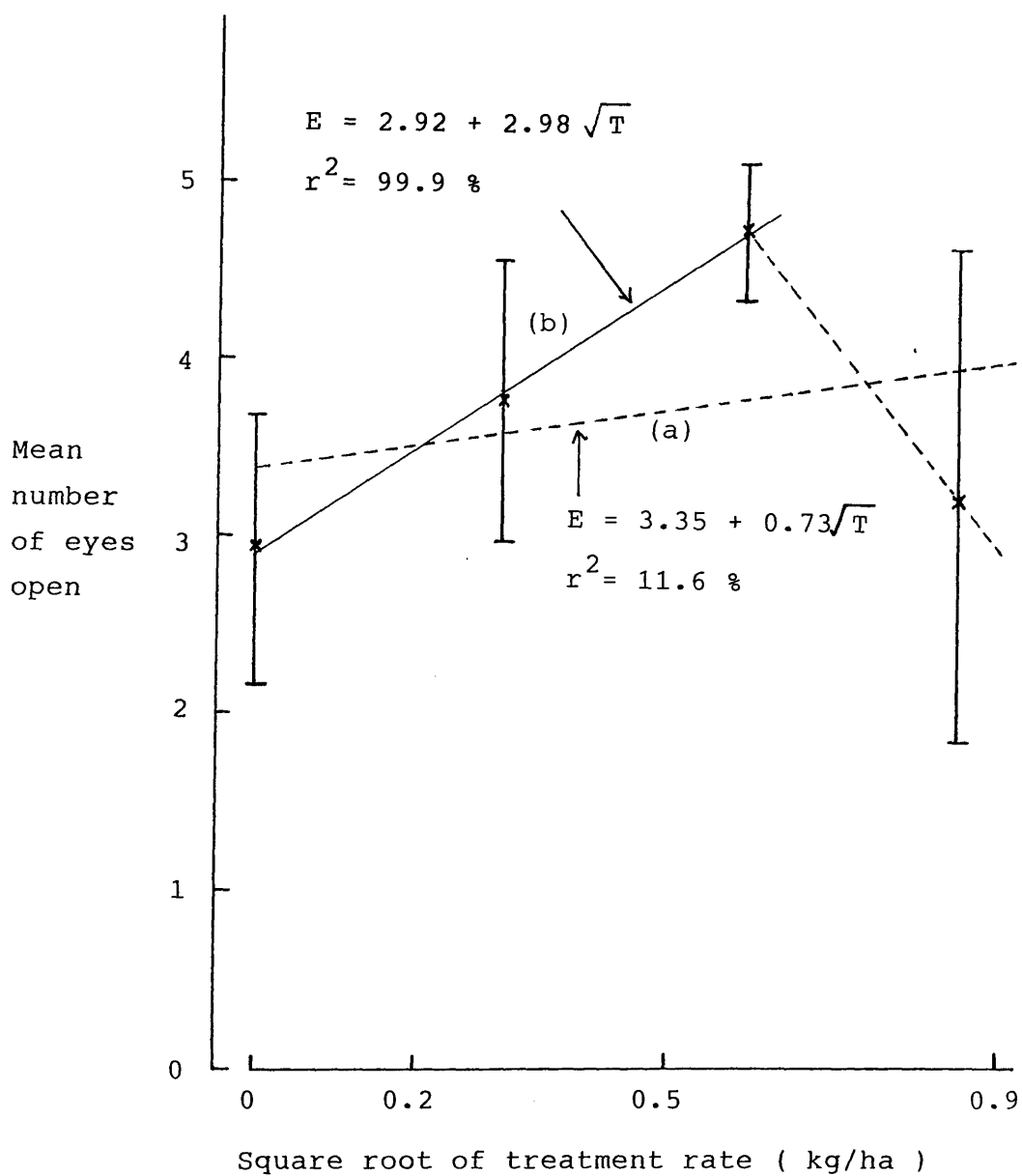


Figure 3.3 Graph of number of eyes open against square root of treatment rate.

x - mean with associated \pm s.d .

On the whole, without the complication of disease and rotting, the number of eyes open would be increased by increasing the treatment level. Number of eyes open can be used for estimating damage effect by glyphosate at the rate of 0.36 kg/ha or less (figure 3.3(b)). This relationship, however could not be used for the damage caused by the glyphosate at a rate higher than 0.36 kg/ha.

3.3.3 Disease or rotting effect.

The results are presented in table 3.5.

Two way analysis of variance showed that the treatments had a highly significant effect while the blocks have no significant effect on disease or rotting of potato tubers over the storage period.

Compared with other stored products such as cereal, potatoes are extremely susceptible to damage by micro-organisms. The rotting caused by fungi and bacteria can result in great losses (Twiss and Jones, 1965). Development of rots caused by a fungus is generally much slower at lower temperature and is almost inhibited completely at 5°C (Burton, 1966). Overall minimising of fungal rots normally demands a temperature of between 5 and 10°C.

Like fungal infections bacterial soft rots also have a higher optimal development temperature around 25 - 30°C. They do, however, develop slowly at 5°C.

Low storage temperature ca. 5 °C which was imposed to avoid sprouting and water loss, encourages gangrene and skin spot (Hide and Lapwood, 1978). The optimum

Table 3.5 (A). Potato tuber, disease effect grouped by treatment and block

Treatment	Disease effect ^a				Treatment mean	s.d. ^b
	Block					
	I	II	III	IV		
A - control	0.00	0.04	0.12	0.00	0.04	0.04
B - 1/16 dose	0.04	0.00	0.16	0.08	0.07	0.07
C - 1/4 dose	0.96	0.60	0.40	0.52	0.62	0.24
D - 1/2 dose	2.20	1.72	1.20	0.76	1.47	0.62

a - average of 25 tubers

b - standard deviation

(B). Analysis of variance

Source of variation	df	Sum of squares	Mean sum of squares	Observed F	Tabulated F	
					p 0.05	0.01
Treatments	3	5.367	1.789	17.78 **	3.86	6.99
Blocks	3	0.458	0.153	1.51 ns		
Error	9	0.912	0.101			
Total	15	6.738				

** = highly significant difference (p<0.01)

ns = not significant

temperature for the development of gangrene (caused by *Phoma* species) is in the range of 0 - 5°C.

From above information it could be said that experimental potato tubers had been stored under conditions which would minimise the effect of rotting by fungi and bacteria. So it would be expected that the control tubers would have little disease or rotting.

Significant differences between treatments were calculated by using Duncan's new multiple range test as mentioned earlier. It was calculated that the significant differences at various span r ranked sample averages as follow :

r	value
2	0.51
3	0.53
4	0.54

From the above value, it could be seen that for the disease or rotting effect, all the treatments were significantly different except treatment A and B where the difference was not significant. A rate 0.09 kg/ha did not give a significant increase of disease or rotting rating compared to the untreated one. Application of glyphosate at the rate higher than 0.09 kg/ha produced a significant increase of disease or rotting rating.

Although there was no statistically significant effect between the blocks on disease or rotting, slight variations between the blocks were expected as mentioned in section 3.3.1 and 3.3.2 i.e. there might be an uneven

distribution or translocation of compound to the tubers and also different sizes and weight of tubers between the blocks.

A plot of disease or rotting against treatment levels is shown in figure 3.4. The regression equation for this graph is :

$$D = - 0.0505 + 2.05 T$$

where

D = disease or rotting rating

T = treatment level (kg/ha).

with an r^2 value of 98.6 %. Other transformations such as log, square root and minus reciprocal were also tried but they did not produce a better relationship. There was a good relationship between disease or rotting and treatment levels. Higher glyphosate treatments produced tubers which were less resistant to disease or produced tubers which were prone to rotting.

3.3.4 Glyphosate residues in treated potato tubers.

A survey of the literature shows that there is no information available on whether glyphosate undergoes degradation in a potato plant or tuber. On the assumption that glyphosate undergoes no degradation after it is translocated to tubers [studies on several species have failed to show any degradation of ^{14}C -glyphosate for periods up to 90 days after leaf application (Claus and Behren, 1976; Gootrup et al., 1976; Putnam, 1976; Sprankle et al., 1975; Zandstra and Nishimoto, 1977)] glyphosate will

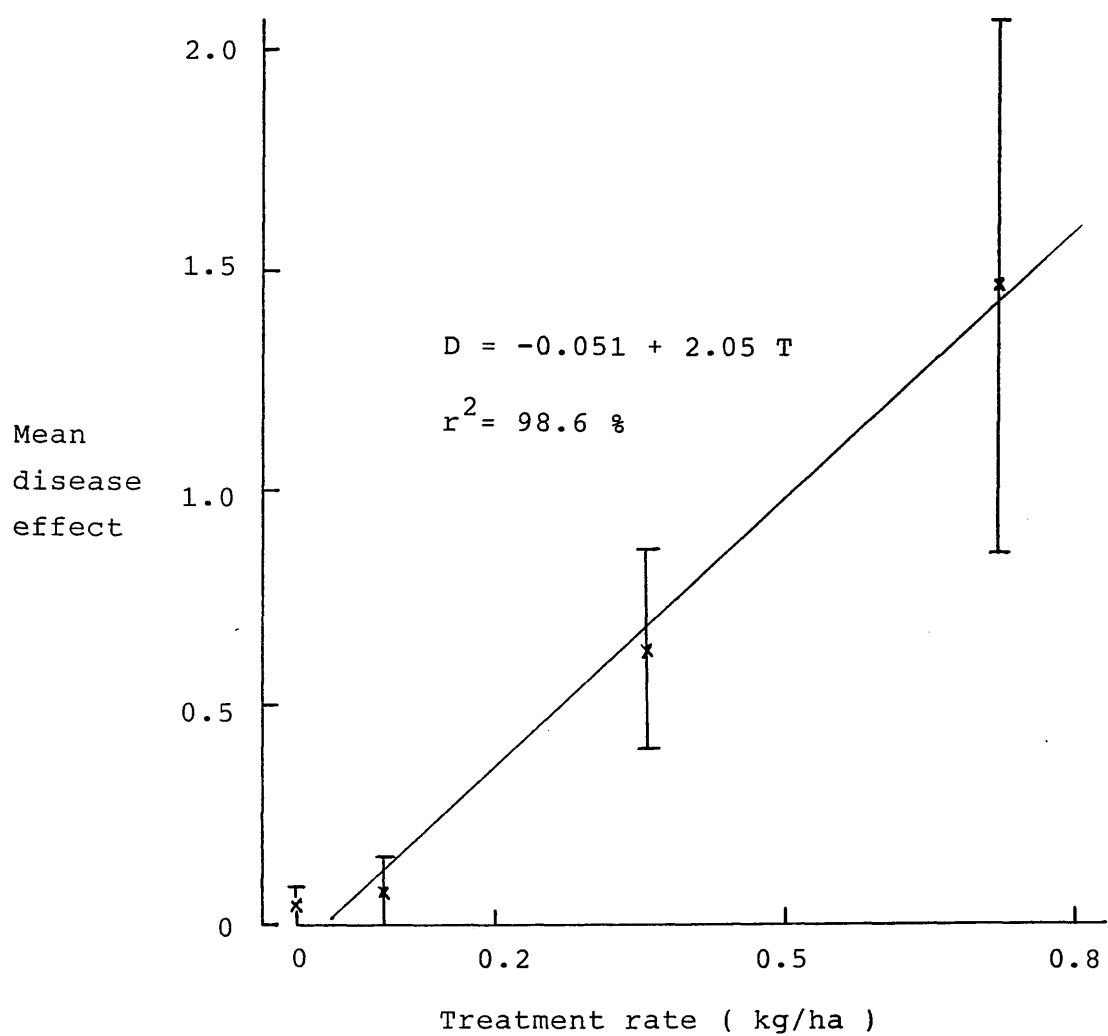



Figure 3.4 Graph of disease effect against treatment rate.

 - mean with associated \pm s.d .

remain stable in the tuber without any loss under storage conditions because of its very low vapour pressure and very high boiling point.

Most of the authors used bio-assay techniques or ^{14}C -glyphosate to study the effect of glyphosate residues especially on the performance of treated food crop commodities such as potato (Smid and Hiller 1981; Putnam, 1978), barley (O'Keefe, 1981b). These two techniques have the disadvantage that they do not determine the actual amount of glyphosate residue in that food crop. Radio labelled techniques would only be accurate if there were no metabolites formed containing the labelled atom.

Glyphosate residues in potato tubers are presented in table 3.6. All the results have been corrected according to recoveries tested in chapter 2.

Two way analysis of variance showed that the treatments have highly significant effects while the blocks have no significant effects on glyphosate residue in potato tuber.

Significant differences between treatments were checked by Duncan's new multiple range test as mentioned earlier. It was calculated that the significant difference at various span r ranked sample averages are as below :

r	value (mg/kg)
2	0.44
3	0.46
4	0.47

Table 3.6 (A). Glyphosate residue in potato (mg/kg)
grouped by treatment and block.

Treatment	Glyphosate residue (mg/kg) ^a				Treatment	s.d. ^b
	Block				mean	
A - control	0.02	0.00	0.009	0.11	0.03	0.03
B - 1/16 dose	0.07	0.13	0.22	0.15	0.14	0.05
C - 1/4 dose	1.01	0.79	0.61	0.36	0.69	0.27
D - 1/2 dose	1.92	1.83	1.47	0.76	1.50	0.52

a - mean of duplicates

b - standard deviation

(B). Analysis of variance

Source of variation	df	Sum of squares	Mean sum of squares	Observed F	Tabulated F	p	0.05	0.01
Treatments	3	5.3478	1.7826	23.2 **	3.86	6.99		
Blocks	3	0.3893	0.1298	1.69 ns				
Error	9	0.6906	0.0767					
Total	15	6.4277						

** = highly significant difference (p<0.01)

ns = not significant

From the above values, it was found that for glyphosate residue in tuber, all the levels of treatments were significantly different except for the treatment A (control) and B (1/16 dose).

Although there was no significant effect between the blocks, slight variations of residues between the blocks were obtained. The probable sources of this variation have been discussed earlier.

The relationship between glyphosate residue and treatment levels can be seen in figure 3.5. The regression equation for this graph is :

$$R = - 0.0164 + 2.07 T$$

where

R = glyphosate residue (mg/kg)

T = treatment (kg/ha)

with r^2 is 99.6 %.

A good relationship was obtained between glyphosate residue and rates of treatment.

While determining glyphosate residues in potato samples, preliminary observations showed that the glyphosate major metabolite, aminomethyl phosphonic acid (AMPA) did not occur in the sample extract by simply comparing retention times in the extract with the AMPA standard.

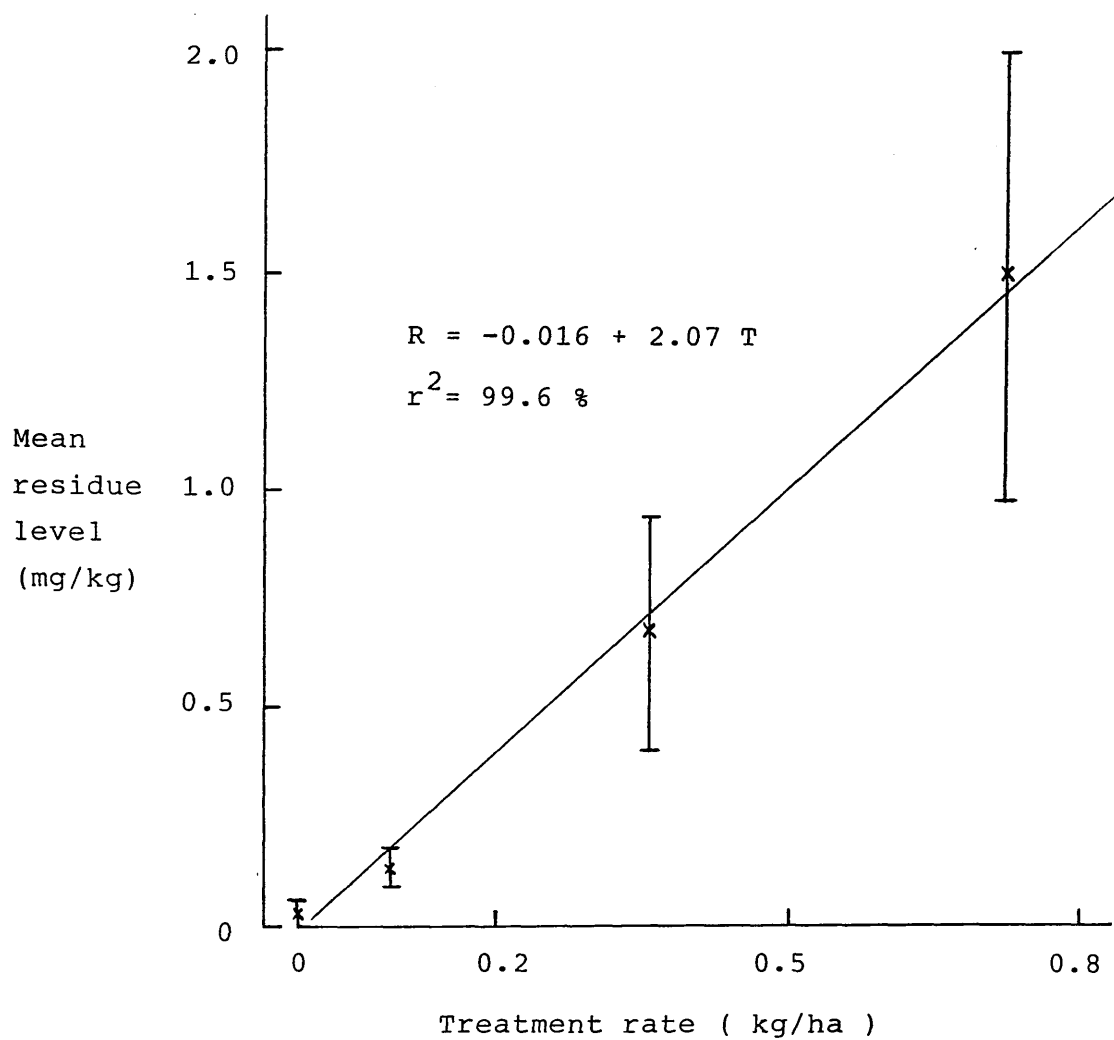


Figure 3.5 Graph of glyphosate residue against treatment rate.

* - mean with associated \pm s.d .

From above results, the determination of the disease or rotting rating or the glyphosate residue in the tubers could be used as indicators of whether the potato plants had been contaminated by glyphosate drift. However, the general level of disease in that particular crop must be determined beforehand (this is not easy to carry out in practice). Other parameters such as sprout length and number of eyes open also could be used but with lesser accuracy.

Attempts were also made to examine the relationship between sprout length, number of eyes open, disease or rotting rating with glyphosate residue using various transformations such as square root, log and minus reciprocal. Again, the relationship between sprout length and glyphosate residue, number of eyes open and glyphosate residue gave almost the same results as these parameters tested against the rate of treatments as obtained earlier. Only glyphosate disease effect and glyphosate residue produced a good relationship with $r^2 = 99.7 \%$ (figure 3.6).

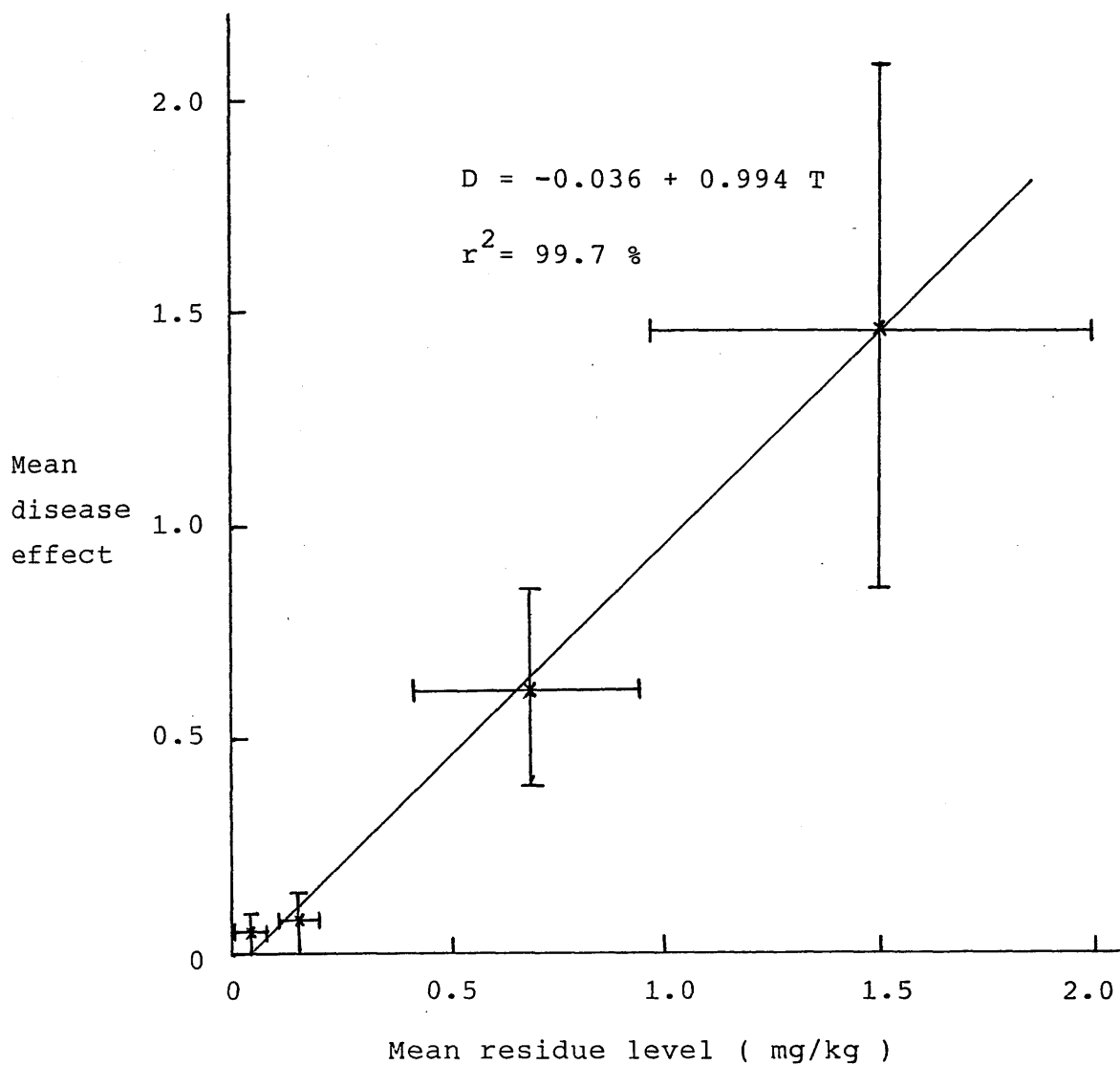


Figure 3.6 Graph of disease against glyphosate residue.

- $\begin{array}{|c} \hline \text{I} \\ \hline \times \\ \hline \text{I} \end{array}$ - mean with associated \pm s.d. for disease.
- $\begin{array}{|c|} \hline \times \\ \hline \end{array}$ - mean with associated \pm s.d. for residue.

3.3.5 Subsequent field performance of glyphosate treated tubers.

Results are presented in table 3.7.

Table 3.7 Field growth performance of glyphosate treated tubers.

Treatment level (kg/ha)	% Produced plants		
	healthy	unhealthy	none
A - control	96	4	-
B - 0.09	32	40	28
C - 0.36	-	4	96
D - 0.72	-	-	100

The lowest rate of treatment produced only 32 % healthy and 40 % unhealthy plants compared to 96 % healthy and 4 % unhealthy for the untreated one. Increasing the rate to 0.36 kg/ha only yielded 4 % of unhealthy plants and 96 % did not grow at all. By applying glyphosate at 0.72 kg/ha yielded no growth of plant.

The above observations were similar to results that had been obtained by Lutman & Richardson (1978) where they found that potato tubers deformed by glyphosate were not capable of forming healthy sprouts and plants.

Although from earlier observation it was found that a treatment rate at 0.09 kg/ha was not significantly different from controls in terms of tuber sprout length (section 3.3.1), number of eyes open (section 3.3.2),

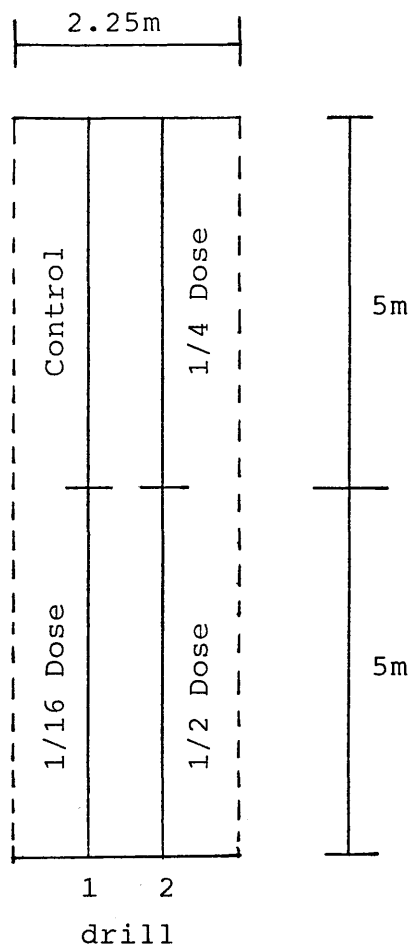


Figure 3.7 Plot layout of subsequent field performance of glyphosate treated tubers.

disease or rotting effect (section 3.3.3) and residue level in the tubers (section 3.3.4), the field growth experiment showed that about only 1/3 of the tubers that had been treated at this level grew healthily compared with controls. This phenomenon was also observed by Lutman & Richardson (1978) where they found lack of symptoms on tubers did not necessarily mean they were not affected by the chemical as sometimes, apparently undamaged tubers failed to form healthy plants.

Although at low level (0.09 kg/ha) treated tubers physically apparently did not show any significant difference compared to untreated ones, a difference in their growth performance was obvious.

Growth performance of these treated tubers was further confirmed when the plants were harvested. Almost all the untreated tubers produced ware tubers (> 57 mm), 0.09 kg/ha treated tubers produced mostly small tubers and the 0.36 and 0.72 kg/ha treated tubers did not produce any tubers.

A photo of the field growth performance for the above experiment can be seen in plate 3.1 showing the degree of blanking obtained.

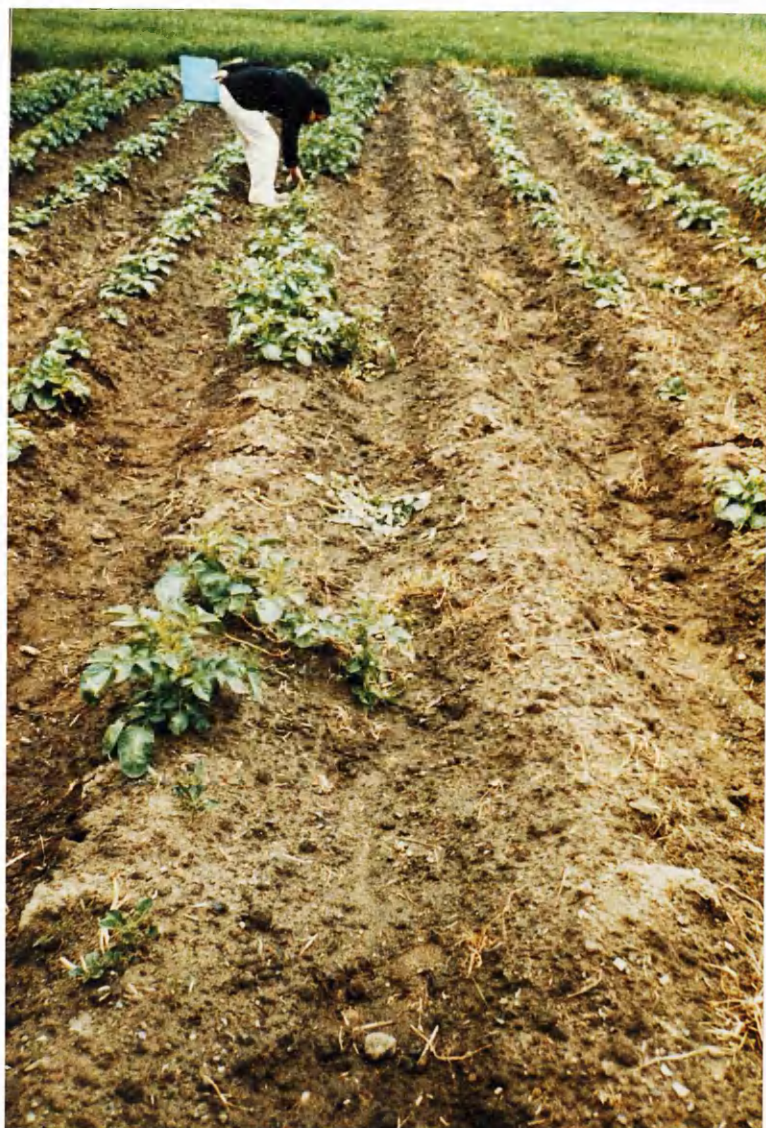


Plate 3.1 Photo of field growth performance of glyphosate treated tubers. Rate of glyphosate treated key as in figure 3.7 .

CHAPTER 4.

ANALYSIS OF GLYPHOSATE IN CEREAL GRAIN (BARLEY).

4.1 INTRODUCTION.

Numerous methods have been developed to determine glyphosate from specific matrices (Moye and St.John, 1980; Guinivan et al.,1982; Roseboom and Berkhoff, 1982; Moye et al.,1983) but many of these methods have been applied primarily to matrices that contain a high percentage of water and by experience, have proved to be easier clean-up challenges (Cowell et al.,1986) .

A method of analysis for glyphosate in potato which contains 75 - 80 % water (Burton,1966) has been developed in chapter 2. Cereal grain contains about 11 - 14 % water and barley an average of 11.7 % water (Belitz and Grosch,1987). As could be imagined this will present different analytical problems from potato because the lower water content will likely contain more impurities in the extract than the extract of higher water content crops.

Cowell et al.,(1986) found that with 5 matrices tested; soybean (grain,legume), grape(fruit), cabbage (vegetable), alfalfa (forage) and fresh water, soybean was considered as a traditionally difficult matrix to analyse and produced the greatest variation in recovery.

Application of glyphosate has been recommended before the cereal crop is harvested (Elliot and Wilson, 1983). In certain situations, the control of weeds with glyphosate in stubble has been found to give low levels of efficacy (O'Keefe and Makepeace, 1985). This is because in late harvested crops or under very dry conditions, the time taken for weeds to grow after harvest may be too long, overlapping with the time for sowing winter cereal or with the onset of cold weather conditions. To overcome these difficulties, application of glyphosate to the standing crop of small grain cereals has been used as a common practice and at the same time acts as a dessicating agent. Barley in Scotland is commonly treated in this manner (see chapter 3).

When cereal grains reach their maximum dry matter content, the moisture content is about 37 % (Mitchell et al., 1980). At this stage the grain matures and exists independently of the rest of the plant. When glyphosate is applied at this stage the risk of reducing crop yield or damaging the grain has been found to be negligible (O'keeffe, 1980).

Application of glyphosate between 7 and 17 days prior to harvest at rates 0.36 - 4.32 kg/ha or between 8 - 10 days at rates 2.16 - 8.64 kg/ha did not affect the grain yields (O'keeffe, 1980, 1981a, 1981b) but slight germination depression was reported when the grain was treated pre-harvest with application rates of 1.44 kg/ha (Sheppard et al., 1982).

It had been reported that at the rate of application of 1.44 or 2.88 kg/ha at pre-harvest, there was no effect on grain quality in barley and wheat or on their subsequent uses for bread-making or malting (O'Keeffe, 1981b). However when poor weather conditions lead to the lodging of treated crops, glyphosate may be absorbed into the cereal husks where the levels vary considerably over a period of 7 - 28 days after glyphosate treatment. In barley, where lemma and palea were not shed during threshing, higher residue levels were recorded (Laermann and Lundehn, 1980).

Maximum permissible limit for glyphosate in barley which falls under grain crops or grain products has been set quite low, at 0.1 ppm because barley products are eaten in large quantities (EPA, 1982b).

In barley glyphosate did not undergo any degradation in the period of three days after application (O'Donovan and O'Sullivan, 1982).

The methods really tested for determination of glyphosate in barley were by the polarographic method at fortification level of 2 mg/kg (Friestad and Bronstad, 1985), gas chromatograph at 0.05 mg/kg (PAM, 1977) and HPLC post-column derivatization at 1 mg/kg (Tuinstra and Kienhuis, 1987).

Most of the methods used to assess the effect of glyphosate applied to the grain as mentioned above were by using bio-assay techniques. It did not determine the actual content of glyphosate in grain. This technique was adopted mainly because of the difficulties in analysis.

From the above information, especially as glyphosate has been used in pre-harvest cereal crops, it is necessary to develop a method of analysis for grain or at least to simplify the existing methods.

Glyphosate is structurally similar to naturally-occurring compounds like amino acids and shows high polarity. For this reason its determination at residue levels is very difficult (Roseboom and Berkhoff, 1982). Gathering this information and other information as stated in chapter 2, the main task to develop a new method or to simplify the existing one lies in the clean-up procedures.

The aim of this work is to simplify the existing method of analysis of glyphosate in cereal grain especially in clean-up procedures by using barley as test specimen so that hopefully the level of glyphosate in other cereal grains also could be monitored quite easily.

4.2 MODIFICATIONS TO THE EXISTING METHOD OF ANALYSIS.

Barley grain used in this experiment was purchased from local shops.

4.2.1 Detection.

Detection by HPLC with pre-column treatment was preferred. The advantage of using HPLC and a pre-column procedure was discussed in chapter 2. The method followed was as mentioned in that chapter. All the sample extracts were adjusted to pH 11 with potassium carbonate before derivatization.

4.2.2 Extraction.

To suit the methods of clean-up, two methods of extraction were adopted :

- i. Blending with a mixture of water and chloroform (75:37 v/v) (Moye and St.John,1980; Moye et al., 1983; Seiber et al.,1984).
- ii. Blending with a mixture of 0.1 M HCl and chloroform (75:37 v/v) (Cowell et al.,1986).

Method i. Blending with a mixture of water and chloroform.

15 g barley, 75 ml water and 37 ml chloroform were placed in 500 ml stainless steel jar. Fortification for glyphosate was performed at this point for recovery. The mixture was blended at medium speed for 10 minutes (Ato Mix blender, MSE, U.K.). Jar was rinsed with 2 X 20 ml water. The contents including the water used for rinsing were placed in 250 ml centrifuge bottle. After centrifugation at 10,000 rpm at 4°C for 20 minutes (MSE 25 high speed centrifuge, U.K.), the supernatant was filtered through Whatman No.1 filter paper into a 500 ml round bottom flask, followed by 2 X 20 ml water used for rinsing the centrifuge bottle. Rotary evaporation was carried out at a temperature below 40 °C to reduce the volume to about 5 ml and stored in the refrigerator for further treatment and analysis.

Method ii. Blending with mixture of 0.1 M HCl and chloroform.

15 g barley, 75 ml 0.1 M HCl and 37 ml chloroform were placed in 500 ml stainless steel jar. Fortification for glyphosate was performed at this point for recovery. The mixture was blended at high speed for 1 minute. Jar was rinsed with 2 X 20 ml water and the contents and the water used for rinsing were placed in a 250 ml centrifuge bottle. After centrifugation at 10,000 rpm at 4°C for 20 minutes, the mixture was filtered through glass wool, followed by 2 X 20 ml 0.1 M HCl for rinsing the centrifuge bottle. The solution was diluted to about 350 ml with deionized water for further analysis.

4.2.3 Clean-up procedures.

4.2.3.1 Using Bond-Elut ion exchange cartridges.

For this experiment 100 mg sorbent and 1 ml volume cartridge was used. Procedures to make the cartridge ready to receive the samples for clean-up were as mentioned in chapter 2.

Sample from extraction method i above was adjusted to pH 12 and diluted to 25 ml with water solution of pH 12. 1 ml of this solution was passed through the cartridge. The eluate was collected. The cartridge was then washed with 1 ml of deionized water and the eluate collected. Finally the cartridge was eluted with buffer pH 1 and the eluate was collected. All the eluates were adjusted to pH 11 with potassium carbonate prior to derivatization and

determination by HPLC pre-column procedure as mentioned in chapter 2.

4.2.3.2 Using chelating and anion exchange resins.

Method by Cowell et al., (1986) was adopted.

Column preparation.

The glass columns dimension 1.9 cm i.d. X 25 cm were used for both columns.

Chelex 100 (50 - 100 and < 400 mesh, sodium form) was obtained from Biorad, U.S.A. Anion exchange resin AG1 -X8 (200 - 400 mesh chloride form) was obtained from B.D.H, U.K.

The Chelex 100 resin was converted to the Fe(111) form by mixing 0.9 kg of resin in a total aqueous volume of 3 litre and 50 ml of 6 M HCl was added followed by 1 litre of 0.1 M ferric chloride solution. The resin was allowed to settle and the aqueous phase was decanted. 2 litres deionized water and 500 ml of 0.1 M ferric chloride were then added to the resin, mixed and the aqueous phase was decanted. This wash was done twice and the resin was then transferred to a large glass column with fritted disk support and rinsed with 4 litres of 0.02 M HCl. The resin was stored at room temperature in a glass bottle under deionized water until used.

(a). Chelex 100 clean-up column.

15 ml Chelex 100 resin in Fe(111) form was transferred to a column containing 7 - 8 ml deionized water. The prepared sample from extraction method ii above was applied to the column and eluted at a rate of

6 - 8 ml/min or less. After sample elution, the wall of the column and resin bed were rinsed with approximately 50 ml deionized water. The column was rinsed additionally with 100 ml of 0.2 M HCl with a wide open stopcock. All eluates were collected for determining the glyphosate content (in original method by Cowell et al., (1986), all these eluates were discarded). All the volumes of these eluates were reduced to about 5 ml on a rotary evaporator. The column was then eluted at a rate of 4 ml/min or less with 22 ml of 6 M HCl solution, the last 15 ml of which was combined with 10 ml of concentrated HCl and retained for anion exchange clean-up. Several fractions of 15 ml 6 M HCl + 10 ml concentrated HCl were also passed through the column to check whether earlier elution solution could elute all of the glyphosate from the column.

(b). Anion exchange clean-up column.

The column was prepared by adding 7 - 8 ml of deionized water and approximately 7 ml of AG1 - X8 anion exchange resin. The column was rinsed with 15 ml of 6 M HCl solution just before applying the sample. The eluate from the Chelex 100 column was applied with the wide open stopcock, and the sample container was rinsed with 2 ml of 6 M HCl onto the column and then 8 ml of 6 M HCl solution was applied. The eluates were collected and concentrated to a volume of about 5 ml on a rotary evaporator.

All the concentrated eluates from Chelex 100 and anion exchange columns were adjusted to pH 11 with 10 M KOH and then diluted to 10 ml with deionized water

solution of pH 11. Glyphosate content in these eluates was determined by HPLC pre-column procedure as mentioned in chapter 2.

4.3 RESULTS AND DISCUSSION.

4.3.1 Using Bond-Elut ion exchange cartridges.

The advantages of using Bond-Elut cartridges as a clean-up material, if it worked, was mentioned in chapter 2.

The idea of using Bond-Elut ion exchange cartridge as a clean-up procedure in the determination of glyphosate in barley where it failed to retain glyphosate in potato extract (refer to chapter 2) was because barley contains ^{interfering substances due to high} less _A carbohydrate especially starch compared to potato. The average carbohydrate in barley was 72 % (Belitz and Grosch, 1987) whereas in potato it was 19.4 % (Smith, 1968).

Carbohydrates that do not carry any charge would be expected not to interfere with the glyphosate retention in the ion exchange sorbent. In this case glyphosate in barley extract would be expected to be retained quite easily on the Bond-Elut cartridge compared to glyphosate in potato extract.

Using Bond-Elut anion exchange - SAX cartridge.

The performance of Bond-Elut anion exchange cartridge to retain glyphosate in barley extract is presented in table 4.1.

Table 4.1 Performance of Bond-Elut anion exchange, SAX to retain spiked glyphosate in barley at 2.5 ppm level.

Sample pH	% glyphosate ^a		
	in sample eluate (not retained at all by the cartridge	in water washing eluate(retained quite loosely by the cartridge	in collected eluate

12	75.4	5.3	19.3

a - mean of duplicates.

It was found that only about 19 % of glyphosate was retained in the cartridge from the barley extract, although under the same conditions, standard glyphosate in water was almost 100 % retained by the cartridge. However, the performance of Bond-Elut anion exchange cartridge to retain glyphosate from barley extract was

better compared to potato extract, where none of the glyphosate could be retained strongly and about 20 % retained quite loosely by the cartridge from potato extract (see table 2.6).

To explain the above result as to why Bond-Elut anion exchange was not so effective in isolating glyphosate from barley extract, there are many possibilities including those mentioned below:

I. Chemical effects.

- a. Glyphosate reacts with metal cations in barley extract to form complexes. Glyphosate metal ion complexes have less ionic charges compared to glyphosate itself. Consequently these complexes will be less retained by the cartridge. The amount of minerals in barley has been reported at average 2.2 % (Belitz and Gosch, 1987) and the amount of cations that could form complexes with glyphosate are as follows :

Ca - 406, Mg - 1410, Fe - 36.7, Zn - 23.6, Mn - 18.9 and Al - 4.9 ppm (Briggs, 1978).

- b. Barley contains anions which are highly attracted by the sorbent, especially the carboxylic acids and amino acids (formed anions at higher pH as in this experiment). Because of their higher molecular weights compared to ordinary anions, their selectivity to an anion exchange sorbent is also higher, and glyphosate is less retained. The major organic acids in barley have been reported

as below (% of dry matter) (Mac Gregor and Edwards, 1968):

Formic acid - 0.51 , malic acid - 0.15, succinic acid - 0.07 and citric acid - 0.06 %.

The major amounts of amino acids in barley have been reported to be (Kent, 1984; Pomeranz and Robbin, 1971): glutamic acid - 25.5, proline - 14.6, aspartic 6.3, arginine 4.9, phenylalanine - 5.2, valine 4.9 ^{g/100g amino acid recovered} and others like alanine, isoleucine, glycine and lysine which are present in less than 4 g/100g amino acid recovered. The effect of these organic and amino acids will be discussed in more detail in the section dealing with chelating resin.

As some of these cation and anion components are present in all matrices analysed, it was considered very difficult to alter or remove their effect. These chemical influences were not studied further.

II. Physical effects.

A. Viscosity of barley extract.

As has been mentioned earlier barley contains relatively low water and high carbohydrate (starch) contents compared to potato. In the process of extraction of the compound from the grain, it was blended to get the compound extracted from the grain tissue into the water phase. When water, a polar solvent is used to extract a compound from high starch tissue, other problems emerge as compared to extraction by organic solvents. Starch occurs

in granules whose size and shape are characteristic of the plant from which the starch is obtained. When intact, starch granules are insoluble in cold water. If the outer membrane is broken by grinding as in this experiment, the granules swell in cold water and form a gel (Morrison and Boyd, 1966).

It was found that barley extract because of gel formation was viscous and quite cloudy compared to potato extract even after centrifuging at 10,000 rpm for 20 minutes. This viscous solution had physical disadvantages as follows :

- i. It reduced the contact area between the compound and the sorbent, encouraging the compound to pass through the sorbent without retention.
- ii. The flow rate of the extract passing through the sorbent was slow.

B. Frothing problem.

One of the major problems dealing with crop water extracts is frothing during volume reduction by rotary evaporation. One of the compounds which contributes to the frothing problem is protein (Sharp, 1987). An antifoaming agent should be used to reduce this problem. In some cases quite a lot of antifoaming agent was needed. It was found that barley extract had higher frothing effect than the potato extract, due to its higher protein content, average of 10.6 % in barley (Belitz and Grosch, 1987) and 2 % in potato (Smith, 1968) and also due to the viscosity of the extract. In this experiment octanol was used as the antifoaming agent.

From the above observation, there was a possibility to improve the retention of glyphosate in Bond-Elut cartridge by eliminating or reducing the physical effects. These have been studied further.

(a). To reduce the frothing problem.

As protein was one of the main factors to frothing, a suitable compound that could adsorb protein in aqueous solution was to be found. From BDH catalogue (1987/88) it was found that the polymeric adsorbent beads, Amberlite XAD-2, a synthetic cross-linked polystyrene polymer without ionic groups is designed for adsorbing water soluble organic substances especially protein in column or batch operation. Amberlite XAD-2 has been tried to reduce the frothing problem and at the same time might reduce the impurities interfering in glyphosate determination because it could adsorb some organic substances.

Procedure : Amberlite XAD-2 was made free from chloride by washing with distilled water before use. 10 g of beads were added to the extract solution(after being centrifuged and filtered) and shaken for 15 minutes. The mixture was allowed to settle and the solution was filtered through Whatman No.1 filter paper with a few millilitres of deionized water for washing.

Result : It was found that Amberlite XAD-2 could reduce the frothing problem for barley extracts significantly. The solution after being treated with Amberlite XAD-2 needed less antifoaming agent for volume reduction by rotary evaporation.

The sample extract after Amberlite XAD-2 treatment was also evaluated for its possibility to reduce the interferences in glyphosate determination in barley extract. This solution was cleaned-up by Bond-Elut anion exchange procedure. It was found that Amberlite XAD-2 treatment did not give any significant interference reduction in determination of glyphosate in barley extract compared to untreated one. This meant that Amberlite XAD-2 adsorbed compounds that were not interfering in glyphosate determination but did help to reduce the frothing effect.

(b). Viscosity of barley extract.

Barley swelled and formed a gel in the extraction process because of its high starch content. As was mentioned earlier this viscous solution reduced the contact between glyphosate in the solution and the ion exchange sorbent. Starch does not swell in organic solvents such as acetone and methanol. Therefore the gel in barley extract solution could be precipitated by adding organic solvent.

In the derivatization process of glyphosate with FMOCCl, a 1:1 (v/v) water-acetone condition at pH 11 was used. Because these conditions were proved not to affect glyphosate solubility, it was decided to use this mixture first in an attempt to remove or precipitate the starch and therefore reduce the viscosity of the extraction solution.

Procedure : The extract after being centrifuged and filtered as in extraction procedure, was adjusted to pH 11. Then acetone was added to make the volume to about 1:1 water-acetone. The mixture was stirred for a few minutes and then filtered through Whatman No.1 filter paper to remove the gelatinous precipitate. Then the volume was reduced and other treatments were as mentioned earlier.

Clean-up was performed by using a Bond-Elut anion exchange cartridge.

Result : It was found that the above method could reduce significantly the viscosity of the barley extract. Before the treatment, the extract could hardly pass through Whatman No.1 filter paper but after the treatment it could pass quite easily through this type of filter paper. At the same time this treatment could also reduce the frothing problem as had happened to untreated extract. To reduce the volume of this solution, very little anti-foaming agent was needed.

The result of Bond-Elut anion exchange performance to retain glyphosate from barley extract after viscosity reduction treatment is shown in table 4.2.

Table 4.2 Performance of Bond-Elut anion exchange
 cartridge to retain spiked glyphosate in
 barley at 1 ppm level, after viscosity
 reduction treatment.

Sample	% glyphosate ^a		

pH	in sample eluate (not retained at all by the cartridge)	in water washing eluate(retained quite loosely by the cartridge)	in collected eluate

12	10.3	42.3	28.8

a - mean of duplicates.

The results from table 4.2 show that
 viscosity of the extract greatly affects the retention of
 glyphosate on the anion exchange sorbent. The amount of
 glyphosate that was not retained by the cartridge
 decreased substantially from 75.4 to 10.3 %. Most of the
 increase in retention was, however, due to loosely
 retained glyphosate which could easily be removed by
 elution with H₂O. The percentage of strongly held
 glyphosate improved from ca. 19 to 29 % but was enough to
 allow the use of the Bond-Elut SAX cartridge as a
 clean-up.

Other treatments especially the chemical ones which had not been tried in chapter 2 were explored.

(C). Precipitation of possible interference compounds.

(i). Adjusting the pH.

It was found that when the pH of barley extract was lowered down to 4, a quite significant amount of light brownish precipitate formed. It is possible this method could be used to reduce the impurities causing interference in glyphosate determinations, i.e. as one of the clean-up steps.

Although these precipitated compounds that occur at lower pH but dissolved at higher pH, had not been identified, it is possible that these compounds were long chain carboxylic acids because they precipitated at lower pH in the medium which was lower than their pK values.

Procedure : Barley extract was adjusted to pH 4 with 6 M HCl with stirring. The precipitate was removed by centrifuging at 3000 rpm for 10 minutes. The clean-up procedure was done by using Bond-Elut anion exchange, SAX cartridge.

Chromatograms before and after precipitation by pH adjustment showed that there was little improvement from the point of view of impurities i.e. precipitation did not remove any interfering compounds from the extract solution.

(ii). Chemical method.

In the determination of glyphosate at residue level, one of the main difficulties was glyphosate was structurally similar to naturally occurring compounds - amino acids (Roseboom and Berkhoff, 1982).

From the method of clean-up and detection by HPLC, two points should be considered before proceeding to the method of precipitation of interference compounds by a chemical method :

1. Organic acids interfere in glyphosate retention to the Bond-Elut anion exchange cartridge, promoting elution of glyphosate from the cartridge. This type of interference has been discussed in detail in chapter 2. Although the amount of organic acids in barley is less compared to potato, this effect still exists as barley has quite a substantial amount of malic acid, formic acid, succinic acid and citric acid as mentioned earlier in this section.
2. Interference compounds should contain the primary or secondary amino groups so that they could react with derivatization reagent, FMOCCl to form detectable derivatives and at the same time should contain anionic groups at pH 4 that could be retained by APS hypersil HPLC column. The most probable interference compounds are amino acids because they match these two criteria.

From the above information, one of the main interferences could be eliminated if the amino acids could be removed from the solution. Only primary amino acids should be precipitated as secondary amino acids have a structure like glyphosate and should remain in the solution.

A survey of the literature showed that there was a method to remove the primary amino acids by precipitation and at the same time did not affect the secondary amino acids - an acetoxime (Vogel, 1964).

Procedure : The extract was adjusted to a certain pH (normally pH 5 or lower). The solution was cooled in cold or ice water. Acetone was added slowly. The cold flask was shaken well and left overnight, during which time the oxime should crystallize out. The crystals were filtered at the pump. The solution was then carried through the clean-up procedure by using Bond-Elut anion exchange cartridge.

Result : A small amount of white precipitate was formed, but the chromatogram of the remaining solution showed that it was not so much different from the extract without acetoxime treatment. Precipitation of primary amino acids did not improve the determination of glyphosate. This could mean that either the reaction of primary amines with the acetoxime did not proceed to completion (even a small quantity of primary amino acids would be relatively high compared to glyphosate - 1 ppm or less) or that primary amines were not responsible for the interferences noted in the chromatograms.

Using Bond-Elut cation exchange, SCX cartridge.

Most of the analysts use both anion and cation exchange resins to remove the impurities from glyphosate extract as mentioned in chapter 2. As barley contains amino acids that are normally positively charged at lower pH and glyphosate always exists in anionic form in measured pH (1 - 14), it seems that some amino acids could be retained by cation exchange Bond-Elut cartridges and at the same time would allow glyphosate to pass through without retention because of its difference in charge. By this method, interferences that might come from amino acids could be reduced. Unretained glyphosate would then proceed to the anion exchanger for a further clean-up procedure.

It seemed possible that cation exchange Bond-Elut could be used as one of the clean-up steps.

Procedure : Barley extract, after its pH was adjusted to 4 and the precipitate was removed as in section (C)i, was passed through the Bond-Elut cation exchange cartridge and then washed with one volume of deionized water. The eluates collected were quantitatively adjusted to pH 12, then proceeded to the clean-up procedure using anion exchange cartridge and detection by HPLC pre-column derivatization.

Result: There were some compounds retained on a cation, SCX Bond-Elut cartridge as coloured compound could be observed on this cartridge. However chromatograms before and after this procedure showed no difference.

It seemed that the cation exchange cartridge could not retain the amino acids and due to this the chromatogram with and without Bond-Elut cation exchange treatment gave almost the same result. This observation could be explained by competitive cations in the solution, thus promoting elution of amino acids.

Liu et al.,(1974) had reported the amount of cations in barley. By using this figure, the amount of barley and water used in the extraction procedure and the final volume, the amount of cations could be calculated as follows :

Mineral	amount ^a (ug/g barley)	in sample passed through the SCX cartridge	
		ug	meq
K	5070	3042	0.078
Mg	1410	846	0.071
Ca	406	243	0.01
Na	254	152	0.006
Fe	37	22	0.001
Zn	24	14	0.0006
Total			0.170 meq

a - after Liu et al.,(1974).

It was calculated that the amount of cations in the sample solution which passed through the Bond-Elut cation exchange, SCX cartridge was at least 0.170 meq because other cations like hydrogen ion, Mn, Cu, Al etc were not included in the calculation. Capacity of Bond-Elut cation exchange, SCX cartridge was 0.12 meq (Van Horne, 1985).

From above finding it was clear that the capacity of the cation exchange cartridge was far less than the amount of cations in the sample extract which passed through it. As a result, the positively charged amino acids could not be retained by the cartridge and the chromatograms of the Bond-Elut cation exchange of the treated and the untreated sample would give almost the same result as has been obtained here.

Coloured material retained or adsorbed to the Bond-Elut cartridge would also be the non polar compounds, because the affinity of SCX sorbent is higher for non polar interaction due to the presence of the benzene ring on its surface.

The above experiments also showed that the compounds retained by Bond-Elut cation exchange, SCX cartridge did not interfere with glyphosate determination/detection. As all the attempts to use Bond-Elut anion exchange, SAX and cation exchange, SCX cartridges as clean-up material in determination of glyphosate in barley were unsuccessful, other clean-up procedures were tried.

4.3.2 Using chelating resin column.

By looking at its structure, glyphosate is able to form complexes with metal ions. Recent data on the behaviour of glyphosate as a ligand have indicated that glyphosate acts as a mono or bidentate ligand, coordinating only through the oxygen atom of the phosphonate group. In calcium complex with glyphosate, the unprotonated glyphosate forms double bridges between calcium(II) ions and two protonated glyphosate ions, complexing the octahedral coordination sphere of the calcium (Knuuttila and Knuuttila, 1985).

The stability constant of Mg, Ca, Mn, Cu and Zn 1:1 complexes with glyphosate have been determined by Madsen et al., (1978).

This specific property of glyphosate could be used as a clean-up procedure in the determination of glyphosate in barley.

Results of the performance of chelating resin, Chelex 100 in determination of glyphosate are presented in table 4.3.

Table 4.3 Performance of chelating resin, Chelex 100 to retain glyphosate from barley extract spiked at 1mg/kg level (15 ug in total).

Resin particle size (mesh)	glyphosate (ug) ^a				
	in sample eluate	in water + 0.2 M HCl washing	0-32	32-57	57-82 ml
< 400	8.3	5.2	3.9	11.2	-
50 - 100	14.7	4.7	7.0	1.7	-

a - mean of duplicates

In the above experiment, HCl was used as elution solution in the isolation of glyphosate from the column, because this solvent could be removed simply by evaporation, leaving behind a pure product (Robinson, 1978).

From table 4.3 it was found that the amount of glyphosate in barley extract was about 13 ug which exceeded the glyphosate added for recovery test. This meant that barley grain (blank) contained quite a high amount of glyphosate. The result also showed that the chelating resin, Chelex 100 Fe(III) form was not capable of retaining all the glyphosate in the extract. The finer size of resin retained more glyphosate than the coarser one. The reason for this was that more of finer size was used because columns were packed on a volume basis leading

to more surface area as the extract was passing through it at a slower rate, it had more contact period and as a result it retained more glyphosate than the coarser one.

Although the retention capacity of the resin was not so great the retained glyphosate was held quite strongly as it needed quite a lot of strong acid to elute it from the column.

At the beginning, the sample after chelating resin treatment was passed through the anion exchange resin prior to detection by HPLC. Comparison was made between the sample with and without anion exchange treatment. It was found that the cleanliness of the sample with anion exchange treatment was not so different and it tended to produce less recovery compared to untreated ones. For this reason therefore, the anion exchange treatment was omitted from the clean-up procedure.

The result of the recovery test for the barley sample is as shown in table 4.4. For this test, Chelex 100 with particle size < 400 mesh was used because it retained larger amounts of glyphosate than the 50 - 100 mesh size. The process took a longer time because the flow rate of the sample that passed through the column was slow. Elution volume was collected up to 57 ml (see table 4.3) and the anion exchange clean-up procedure was omitted.

Table 4.3 suggests that complete equilibrium may not have been reached using 50-100 mesh size resin.

Table 4.4 Recovery test of glyphosate in barley.

Level of glyphosate in barley(spiked)	% recovery ^a	
	uncorrected	corrected to blank
1.0 ppm	72	52
0.1	180	42

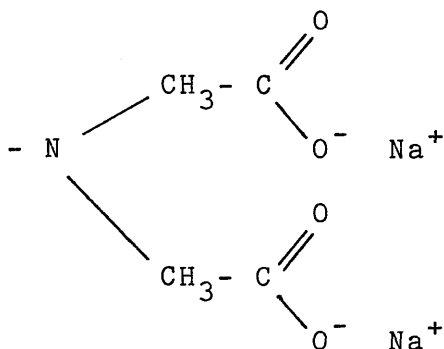
a - mean of duplicates.

The higher level of glyphosate was not tested because its maximum permissible limit in barley, as mentioned earlier, was 0.1 ppm.

The blank sample contained a relatively high amount of glyphosate, therefore it influenced the added (spiked) glyphosate retained by the resin especially at the higher level. If the glyphosate level in the blank was negligible, we could expect the recovery would be higher than 70 %.

This result was adequate compared to what had been obtained by Cowell et al.,(1986) in his 5 interlaboratory analysis of glyphosate in 5 different types of matrices mainly higher in water content than barley, by using almost the same clean-up procedure (with anion exchange clean-up procedure) and the detection by post-column derivatization, they obtained recovery values from 21.4 to 135.5 %.

Chelex 100 resin is composed of weakly iminodiacetic groups on a polystyrene lattice (Bio-rad Laboratory, 1965) in sodium form.



When it was converted to the ferric form, it was bound to iminodiacetate groups by chelating properties. Although part of the Fe(111) was bound to the iminodiacetate, in Fe(111) saturated condition, it still had the capability to chelate with other chelating compounds such as glyphosate.

Although Fe(111) could bind to glyphosate very strongly due to its complexing properties, its complexing power was reduced as part of its atom was complexing with iminodiacetate groups. Therefore we could expect that the complexing power of Chelex 100 in Fe(111) form was less compared to Fe(111) alone.

This phenomenon could be seen clearly from tables 4.3 and 4.4 where the amount of glyphosate retained by the column was less compared to the amount present in the solution.

Retention of glyphosate in the column could also be reduced by other competing ligands or complexing compounds in the extract, especially the polycarboxylic acids. Barley as reported by Mac Gregor and Edward (1968) contains significant amounts of complexing compounds such as succinic acid, malic acid, citric acid etc. Their amounts and relative complexing formations compared to glyphosate with Fe(111) (Madsen et al., 1978; Perrin, 1979; Motekaitis and Martell, 1985) are as in table 4.5.

Table 4.5. Amount of chelating compounds and their chelating power to Fe(111).

Complexing Compound	log complex stability constant	content in barley (ppm).
Succinic acid	$K_1 = 7.89$	700
Formic acid	1.70	5100
Malic acid	10.4	1500
Citric	10.2	600
Iminodiacetic acid (IDA)	10.7	resin active- groups
Glyphosate	16.1	< 1.0

Amino acids such as glutamic acid, proline, aspartic, leucine although existing in small amounts as mentioned earlier, could form complexes with mineral ions (Perrin, 1979).

From table 4.5 it could be said that the chelating power of the IDA - Fe(111) complex to retain glyphosate by chelation had been reduced quite significantly by other chelating compounds. Although these compounds have slightly lower or lower chelating power to Fe(111), their amount was relatively high compared to glyphosate concentration. Therefore we could expect not to obtain a high recovery by using this chelating resin column as a clean-up procedure because it could not retain all the glyphosate in the barley extract especially at higher concentration.

In the above experiment, pH of the extract was between 1.8 - 2.0. Normally the chelating power of chelating compounds could be increased by increasing the pH (Schwarzenbach and Flaschka, 1969), i.e. the anionic charge of these compounds was increased.

There is a possibility to increase the binding power of glyphosate to the resin by increasing the pH of the extract because of the above reason, and more glyphosate could be retained by the resin by this technique.

Procedure : Chelex 100 column Fe(111) form < 400 mesh was prepared as before. Then it was washed with deionized water until the pH of the column was between 3.5 - 4.0. Sample extract after centrifuging, filtration and dilution, was adjusted to pH 4.0. Any precipitate formed

was removed by centrifuging at 3000 rpm for 10 minutes. The sample then was passed through the column. Other treatments in the procedures were as mentioned earlier.

Result : The performance of Chelex 100 Fe(111) form at pH 4.0 in retaining glyphosate from barley extract is presented in table 4.6.

Table 4.6. Performance of chelex 100 Fe(111) form to retain glyphosate from barley extract at pH 4.0.

Level of glyphosate in barley (spiked)		glyphosate retained on the column (ug) ^a	% recovery
ppm	amount added(ug)		
1.0	15	3.4	6
0.1	1.5	2.7	13
blank	0	2.5 *	

a - mean of duplicates.

* - indicated that blank (barley grain) contained certain amount of glyphosate.

To increase the pH of Chelex 100 Fe(111) form to 4, deionized water was used. This process took quite a long time. Attempts to shorten the time by using buffer solution of pH 4 could not be practised because the buffers contained complexing agents such as phthalate,

citrate, acetate and succinate ions (Meites, 1963; Colowick and Kaplan, 1955).

pH 4 was chosen because at this pH, 100 % glyphosate was in monoanionic form compared to 35 % at pH 2.

From table 4.6, it was found that chelating resin, Chelex 100 Fe(III) form almost totally lost its complexing power to retain glyphosate from barley extract at this pH. It retained almost the same amount of glyphosate either from the low level solution of glyphosate or from the higher one.

Increasing the pH of chelex 100 Fe(III) form resin, decreased its ability to retain glyphosate from barley extract.

The above observation could be explained by many possibilities including those mentioned below :

- i. Less amount of iminodiacetate-Fe(III) complex existed in the resin. From the graph of $\log \alpha_{\text{MOH}}$ (ferric hydroxide) vs pH for Fe(III)-iminodiacetate complex (Kragten, 1978) it was found that the ratio of iron(III) released from this complex was increased by increasing the pH.

It was calculated that the ratio of iron (III) released at pH 4 and pH 2 from this complex was 631. Consequently less ferric ion existed in the complex at pH 4 than at pH 2, so the amount of iminodiacetate-Fe(III) complex was also lower. Therefore the retaining power of the column was reduced.

ii. At pH 4 more carboxylic acids and amino acids ionized to anionic form and acted as stronger chelating agents than at pH 2. At this pH these acids competed strongly to the Fe(111) resin complex because they existed in bigger amounts and their pK values were not much lower compared to resin active group, IDA and glyphosate. This condition increased the promotion of the elution of glyphosate from the column or it provided only a small portion of available active space for glyphosate as was shown in table 4.6, where glyphosate from barley extract was retained by the column in almost the same amount either from the blank or higher level of glyphosate.

The above assumption was supported further by comparing the impurities pattern present in the elution solution at pH 2 and 4. In glyphosate eluate (fraction 0 - 57 ml) the impurities present were not much different although the impurities present were slightly higher at pH 4 than 2. In the latter fraction (57 - 82 ml) it was found that a lot of impurity peaks came out in this fraction at pH 4 compared to a few at pH 2. This observation clearly indicated that with impurities having chelating properties, their chelating powers were greatly increased by increasing the pH from 2 to 4. Their binding or chelating power to the Fe(111)-iminodiacetate were increased greatly and they needed a bigger volume of strong acid to elute them from the column. At the same time they greatly

decreased the relative chelating power of glyphosate to this resin.

From the above observation it was found that by using chelating resin as a clean-up material it was quite difficult to assess the recovery test of glyphosate from the barley grain which contained quite high amounts of glyphosate because the Chelex 100 Fe(111) form resin had a limited chelating power and it could not retain high amounts of glyphosate from the barley extract. Attempts were made to purchase barley grain from other shops with the hope that it would contain a low level of glyphosate, but in this period of the experiment, all of the available sacks showed relatively high amounts of glyphosate (ca. 1 ppm).

There is a possibility to increase the amount of glyphosate in the column by increasing the amount or volume of resin used. As the result in table 4.3, with the same volume of resin used, the finer resin (< 400 mesh) retained about 73 % higher than the coarser one (50-100 mesh). The problem was that the flow rate of solution passing through the finer size resin column was too slow compared to the coarser one. If a bigger volume of finer resin is to be used, a longer time would be required. This would not be a practical solution.

Because of the problems mentioned above, other alternative clean-up procedures were explored as follows :

4.3.3 Charcoal as a clean-up material.

As mentioned in chapter 2, charcoal was used as one of the clean-up steps in the determination of glyphosate by gas chromatography. Charcoal column chromatography was also used as a clean-up procedure to determine glyphosate in potato by HPLC as mentioned in chapter 2. Therefore it was worth checking the suitability of charcoal at least as a pre clean-up or as one of the clean-up steps in the determination of glyphosate in barley.

To minimise the effect of the impurities in charcoal, analytical grade charcoal, Norit SX 1G was used. Batch adsorption was preferable for the procedure.

Procedure : Sample treatments such as extraction, centrifuging and filtration were the same as mentioned in section 4.3.2. Then the sample was adjusted to a certain pH. 5 g charcoal was added. The mixture was stirred occasionally for about 30 minutes, then it was filtered. A few millilitres of deionized water was used for washing. The volume of the solution was reduced by rotary evaporator to about 5 ml. pH of this solution was adjusted to 11 with 10 M KOH and diluted to 10 ml with pH 11 water solution. 1 or 2 ml was used for derivatization and detection by HPLC pre-column procedure as mentioned earlier.

Result : The result of above attempt is presented in table 4.7.

Table 4.7 Performance of Norit SX 1G charcoal clean-up in the determination of glyphosate in barley.

Level of glyphosate in barley (spiked)		pH of the solution	recovery ^a	
ppm	amount added (ug)		ug	%
1.0	15	8	4.6	30.7
		12	7.7	51.3
0.1	1.5	8	-	-
		12	very low	very low
blank	-	8	-	-
		12	-	-

a - mean of duplicates.

The pH of the extract solution was adjusted to either pH 8 or 12 because at this condition glyphosate exists in 100 % dianionic and trianionic forms respectively. The relationship between the pH, the molecular charges and the adsorption by charcoal has been discussed at length in chapter 2. At these pH conditions we could expect the adsorption of glyphosate by charcoal will be minimised.

Result from table 4.7 indicated that for 1.0 ppm level, quite high recovery was obtained at pH 12. Lower recovery had been obtained at lower pH 8, as had been expected.

By comparing the results from table 4.7 and the results obtained earlier (table 4.3, 4.4 and 4.6) using the same barley sample, it was found that charcoal Norit SX 1G even at higher pH (=12) adsorbed quite significant amounts of glyphosate. This could be seen when almost 0 ug was recovered from 0.1 ppm spiked and blank sample but the previous experiment showed that the blank sample contained relatively high amounts of glyphosate. This adsorption phenomenon was also observed by Sprankle et al.,(1975b).

Although quite high recoveries were obtained for the 1.0 ppm level, about 51 % at pH 12, it could be guessed that if the same condition was applied to a low glyphosate content barley, a low recovery would be obtained because the charcoal would adsorb more added glyphosate.

The other disadvantage of using charcoal Norit SX 1G as a clean-up material (batch adsorption) for the determination of glyphosate in barley was that, from the chromatogram, it was found that the retention time of the glyphosate-derivative was shifted slightly and retained longer and thus became very close to the next impurity peak. This shift made the quantitation of glyphosate more difficult.

From the above observations, the decision was taken to exclude charcoal Norit SX 1G as a clean-up material or as one of the clean-up steps in determination of glyphosate in barley.

4.3.4 Conclusions.

None of the clean-up methods tested proved to be totally successful in isolating glyphosate with acceptable recovery rate ($> 70\%$). Using ion exchange bonded sorbent cartridges (SCX and SAX) was judged to be the least successful. The batch charcoal allowed the determination of glyphosate when spiked with glyphosate equivalent to 1 mg/kg in barley. However, at lower levels of glyphosate the charcoal adsorbed the majority of glyphosate present and was therefore considered unsuitable unless large amounts of glyphosate were known to be present in the sample.

The chelating resin, could be used in the clean-up of barley extracts for glyphosate determination to a low level (< 0.1 mg/kg) although recovery rates would be ca. 40 % thus affecting the limit of detection possible with this procedure.

Recently, a method for glyphosate determination in barley was reported by Tuinstra and Kienhuis (1987). Their procedure involved a combination of two HPLC columns (anion pre-column and a standard HPLC anion-exchange column) to allowed glyphosate separation from interfering peaks. The method involved post-column derivatization and

resulted in a recovery of 80 % at 1 mg/kg level with a limit of detection of 0.2 mg/kg.

A combination of lack of time and the preference for pre-column derivatization as detailed in chapter 2 meant that this method was not to be investigated further although it remains an alternative to the approach described in this chapter for glyphosate residue determination at ca. 1 mg/kg.

CHAPTER 5.

DETERMINATION OF COMPLEX STABILITY CONSTANTS OF ALUMINIUM AND IRON (111) WITH GLYPHOSATE AND THEIR ENVIRONMENTAL IMPLICATIONS.

5.1 INTRODUCTION.

Glyphosate structurally has three functional groups (amino, carboxylic and phosphonic). Its structure is quite similar to aminopolyacids such as iminodiacetic acid and ethylene diamine-N-methylene phosphonic acid. These compounds were reported to act as chelating agents and formed complexes with metal ions (Perrin,1979). Therefore, we could predict that glyphosate could also form complexes with metals ions. A study of the chelating properties of glyphosate is justified not only for its chemical interest but also because this information is essential for understanding the behaviour of this herbicide in soil and plants.

The 1:1 complex stabilities of glyphosate with metal ions such as Mg, Ca, Mn, Cu and Zn have been determined by potentiometric titration (Madsen et al.,1978). The replacement of one carboxylate group in iminodiacetic acid (IDA) by a phosphonic group slightly increased the coordination ability, however there was not always an increase for aminopoly acids (Westerback et al.,1965). So it could be postulated that complex stability constants of

glyphosate with metal ions will be higher than those of IDA.

In the study of complexes of amino methyl phosphonic acid, which is the metabolic product of glyphosate, with Co(II) (Glowiak et al., 1980a), Cu(II) (Glowiak et al., 1980b) and Zn(II) (Fenot et al., 1978) it was found that the phosphono group coordinated through two oxygen atoms to adjacent metal atoms, forming chains across phosphono bridges. These complexes did not exhibit coordination through the amino group.

By looking at its structure, it can be considered that glyphosate acts as a tridentate ligand. However, from a preliminary study, Knuuttila and Knuuttila (1985) indicated that glyphosate acted as a mono or bidentate ligand, coordinating only through the oxygen atoms of the phosphonate group. This work was further supported by Motekaitis and Martell (1985), who indicated that carboxylate coordination may be dismissed since monodentate carboxylate donors have stability constants around 10 or less ($\log K < 1$).

This supported the earlier finding in investigations with phosphate where Sprankle et al., (1975b) predicted that glyphosate was bound to soil through the phosphonic acid moiety and addition of phosphate decreased its adsorption on soil. Shoval and Yariv (1979), by utilising Fe^{3+} and Al^{3+} -saturated montmorillonite in investigating several glyphosate-cation complexes by infra red spectrophotometry, concluded that there were complexes formed within the inter-layer spaces of the clay

minerals. Close correlation between glyphosate sorption and soil with a high amount of organic matter, iron and aluminium has led some investigators to propose a binding of glyphosate to this soil by an organic matter-metal-glyphosate complex (Nomura and Hilton, 1977; Hensley et al., 1978). Dytyuk et al., (1982) observed that the deposit of mineral salts from aqueous solution could be decreased by using glyphosate or its salt. Shea and Tupy (1984) found that the phytotoxicity of glyphosate in hard water was restored by adding EDTA, a well known chelating agent.

The normal complexes (ML type) probably chelate the metal ion in an approximately planar arrangement, thus minimising charge repulsions between the trans-oriented negatively charged phosphonate and carboxylate groups (Motekaitis and Martell, 1985). From preliminary study of the Ca-glyphosate complex, Knuuttila and Knuuttila (1985) proposed that the unprotonated glyphosate formed double bridges between calcium ions and two protonated glyphosate ions completing the octahedral coordination sphere of the calcium ion (figure 5.1).

Motekaitis and Martell (1985) had determined 1:1 and 2:1 complex stability constants of glyphosate with trivalent metal ions such as aluminium and iron(III) in the presence of $I = 0.10$ (KNO_3). I is the ionic strength and is defined by the equation $I = 1/2 \sum m_i Z_i^2$, where m_i represents the molar concentration of the various ions in the solution and Z_i are their respective charges. The nitrate medium is not a relatively inert or neutral medium for trivalent metal ions. Nitrate could form complexes

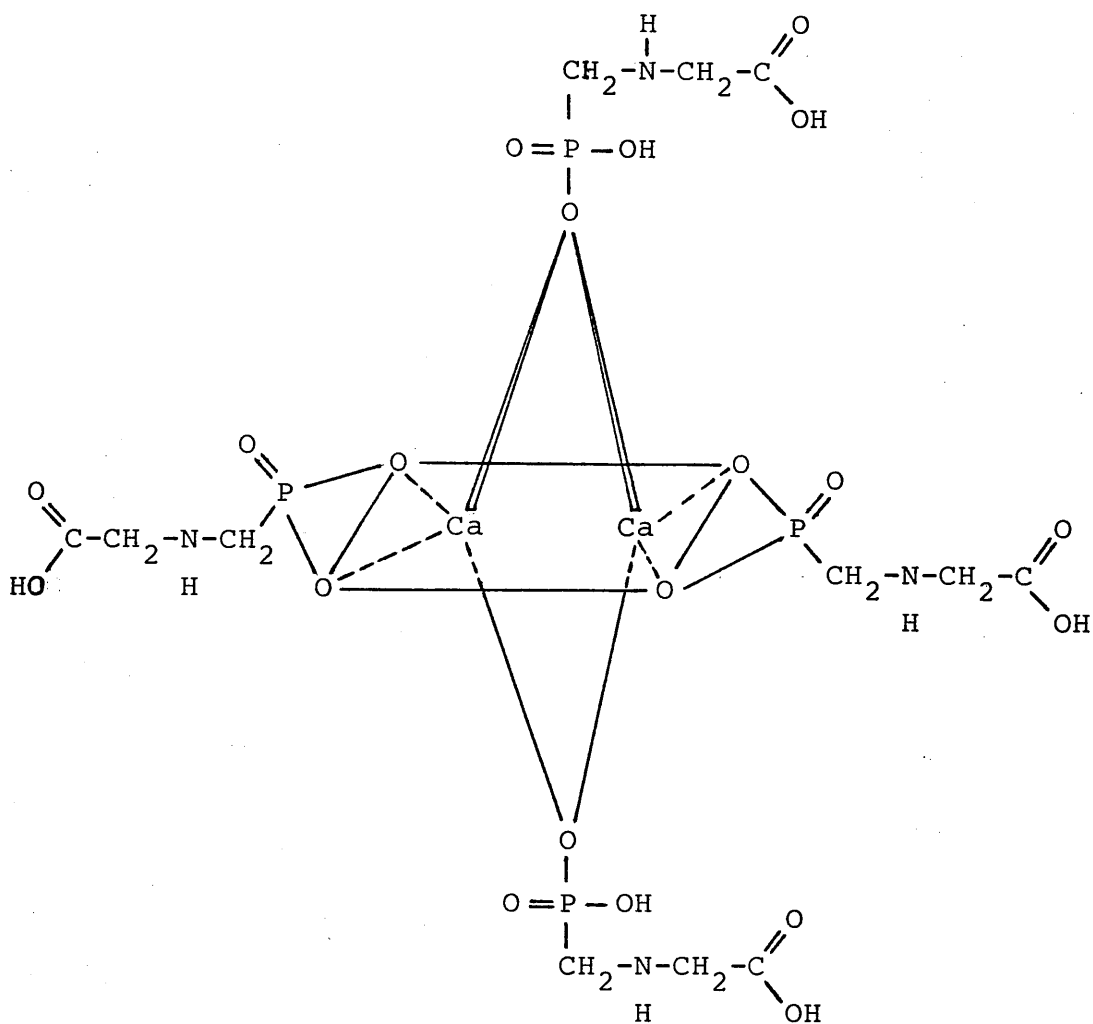


Figure 5.1 Structure of calcium-glyphosate complex (proposed by Knuuttila and Knuuttila (1985) from their preliminary study).

with Al^{3+} and Fe^{3+} ; their complex stabilities are 1.3 and 10.0 respectively (Lindsay, 1979). Perchlorate was shown to be a neutral medium especially for trivalent metal ions where it did not form any complexes with Al^{3+} and Fe^{3+} (Stumm and Morgan, 1981).

However there is no information yet about the complex stability constant of glyphosate and trivalent metal ions in a relatively inert medium such as perchlorate. In order to know the fate of this chemical and environmental consequences especially in soil, more information about these metal-glyphosate complex stability constants should be found because aluminium and iron are major elements in soil; iron contents range from 0.7 to 55 % and aluminium from 1 to 30 % (Lindsay, 1979). As mentioned in chapter 4, it was important to get more information about these metal-glyphosate complexes, especially to develop the method of determination of glyphosate by using chelating or ion exchange resin as clean-up procedures.

Although glyphosate has been shown to bind strongly to clay minerals (Sprankle et al., 1975; Hance, 1976; Hensley et al., 1978; McConnell and Hossner, 1985; Glass, 1987), it still could give rise to environmental problems because the adsorption is often an equilibrium process and binding is therefore reversible (Calvet, 1980).

5.2 EXPERIMENTAL.

5.2.1 Materials.

Aluminium(111) and iron(111) perchlorates were purchased from Aldrich Chemical Co.Ltd. Glyphosate was obtained from British Greyhound, U.K. with purity 97.0 %. Other chemicals were commercially available reagent grades.

All solutions were prepared with boiled deionized water. Standard buffers for calibration were prepared from analytical grade chemicals. Sodium hydroxide 0.05 and 0.2 M were prepared from 1.00 M volumetric solution (Formachem, Scotland).

Standardization of stock solutions.

a. Stock solutions of aluminium perchlorate and iron(111) perchlorate were standardized as follows :

1. Aluminium perchlorate.

Aluminium perchlorate stock solution was standardized using the method by Schwarzenbach and Flaschka (1969).

Reagents : Standard solution of 0.02 M EDTA, 0.02 M ZnSO_4 , acetic acid-ammonium acetate buffer containing one mole of each per litre and dithizone in ethanol.

Procedure : 3 ml solution of ca. 0.1 M aluminium perchlorate and 20 ml 0.02 M EDTA were mixed together, then acetic-acetate buffer was added to bring the pH to 4.5 (checked with pH meter). The mixture was boiled for a short time, cooled and diluted with a volume of ethanol to make it a 1:1 (v/v) ratio. 2 ml of indicator was added for each 100 ml solution and

back titration with 0.02 M ZnSO_4 was carried out. The end point was recognised by a very sharp change of colour from a greenish violet to red.

ii. Iron(111) perchlorate.

Iron(111) perchlorate stock solution was standardized by using AAS (Perkin-Elmer model 1100 B) using the following conditions :

C_2H_2	:	2.5 litre/min.
air	:	8.0 litre/min
λ	:	248.3 nm
slit	:	0.2 nm
lamp current	:	20 mA

Standard iron(111) solution, (Spectrosol) was obtained from BDH chemicals, U.K.

b. Standardization of dilute stock solution of perchloric acid.

Dilute stock solution of perchloric acid was made by diluting 72 % (w/w) perchloric acid (May and Baker, U.K.). It was then standardized with 1.00 M NaOH, using methyl orange as indicator.

c. Preparation of standard buffer solution.

Procedure of Robinson (1987) was adopted, as follows:

Phthalate buffer : 0.05 molal (m) of potassium hydrogen phthalate

Phosphate buffer : 0.008695 m KH_2PO_4 and 0.03043 m Na_2HPO_4

Borax buffer : 0.01 m $\text{Na}_2\text{B}_4\text{O}_7$

Their pHs at 25°C were 4.008, 7.413 and 9.180, respectively

5.2.2 Apparatus :

pH was measured with a Radiometer digital pH meter model PHM 64 (Denmark), using a combined glass electrode (Russel Ltd,U.K.). Temperature was maintained at 25 + 0.1°C with water bath (Baird & Tatlock,U.K.). The solution was stirred by hot plate magnetic stirrer (Corning,U.K.).

5.2.3 Procedure :

All solutions, except standard buffers were adjusted to ionic strength of 0.10 with 0.5 M NaClO₄.

pH calibration : Three standard buffers were used for calibration. Calibration was rechecked after each run of a titration curve.

In order to be able to calculate [H⁺] and [OH⁻] from the pH reading, pH was measured in a series of HClO₄ solutions from 0.1 to 10 x 10⁻³ M and in a series of NaOH solutions from 0.25 to 1.5 x 10⁻³ M.

Recording a titration curve : The initial volume of the titrand solution was 30.0 ml and it was 2.67 x 10⁻³ M with respect to glyphosate. Metal concentration was equal to ligand concentration. A total of 5 ml of 0.05 M NaOH was added and stirred. About 50 points of the titration curve were recorded.

5.2.4 Calculations :

(i). Titration curve without metal :

The method by Madsen et al., (1978) was adopted. Degree of neutralization, n for this type of titration could be calculated as below :

$$n = \frac{B - A + [H^+] - [OH^-]}{C_L}$$

where :

B = amount of strong base added,
(equivalent/litre).

A = excess of strong acid added prior to
titration. In this experiment $A = 0$.

$[H^+]$ = concentration of hydrogen ions in the
solution (mole/litre). Calculated from
measured pH and the actual concentration of
 $HClO_4$

$[OH^-]$ = concentration of hydroxide ion in the
solution (mole/litre). Calculated from
measured pH and actual concentration of
NaOH.

C_L = total concentration of glyphosate
(mole/litre).

As the pK values are widely spaced, they can be calculated simply as follows :

$$pK_1 = pH + \log \frac{1 - n}{n} \quad \text{for } 0 < n < 1$$

$$pK_2 = pH + \log \frac{2 - n}{n - 1} \quad \text{for } 1 < n < 2$$

$$pK_3 = pH + \log \frac{3 - n}{n - 2} \quad \text{for } 2 < n < 3$$

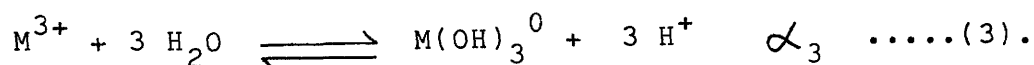
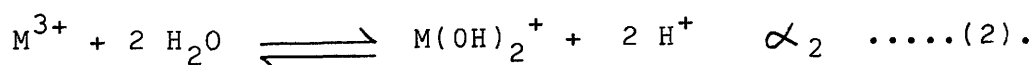
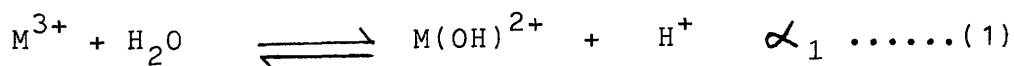
From preliminary study, Madsen et al., (1978) showed that although glyphosate should be able to take up a proton to form a positive ion, the corresponding pK value was negative; this possibility was disregarded in further work. Motekaitis and Martell (1985) also reported that the protonation constant of glyphosate (in L^{-3}) corresponded to the values of above pK_1 , pK_2 and pK_3 . This is supported by crystalline structure (Knuuttila and Knuuttila, 1985) where in solid state, one phosphono hydrogen has shifted to the amino group and thus a zwitterion is formed. In aqueous solution this same hydrogen atom alternates between the phosphono and amino groups at such a high frequency that its location cannot be determined.

(ii). Titration curves with metal ions.

The method by Chaberek and Martell (1952), Madsen et al., (1978) and Lindsay (1979) had to be modified in order to take into account the acid-base properties of the complex, the hydrolysis encountered by the trivalent metal ions in aqueous solution and the formation of hydroxides of these metal ions due to increasing the pH in the titration process, even in acidic media.

Solubility products (K_{sp}) of hydroxides of trivalent metal ions such as aluminium(III) and iron(III) are extremely low, so these hydroxides would form even in acidic media. K_{sp} for hydroxides of aluminium(III) and iron(III) at 25°C were 2.0×10^{-34} (Hogfelt, 1982) and 2.64×10^{-39} (Chang, 1987) respectively.

Trivalent metals ions also undergo hydrolysis in aqueous solution where the following reactions predominate (Kubota, 1956; Lindsay, 1979; Stumm and Morgan, 1981).



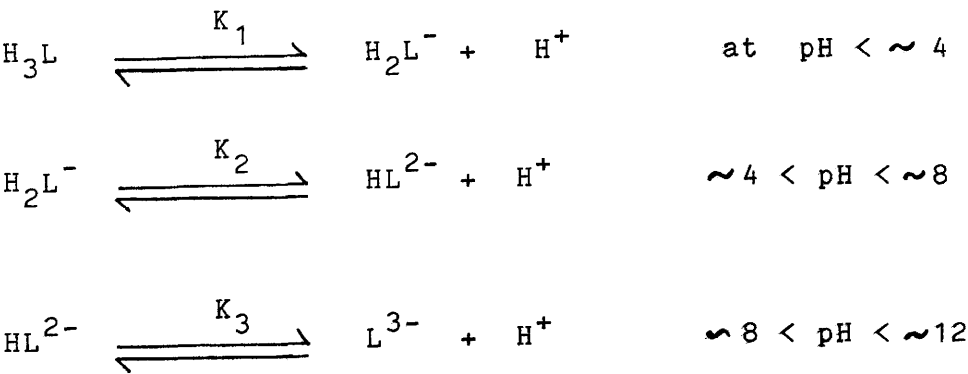
Values for α_1 , α_2 and α_3 for aluminium(III) and iron(III) at 25°C are 5.01×10^{-6} and 6.46×10^{-3} , 5.01×10^{-10} and 2.04×10^{-6} , 1.02×10^{-15} and 8.13×10^{-14} respectively. From these values and data from Aveston

(1965) it is possible to calculate the hydrolysis species of aluminium(111) and iron(111) in aqueous solution at different pHs at 25°C as in table 5.1.

Table 5.1 Hydrolysis species of aluminium(111) and iron(111) at different pHs at 25°C (ratio to M³⁺).

pH	Al(OH) ²⁺	Al(OH) ₂ ⁺	Al(OH) ₃ ⁰	Fe(OH) ²⁺	Fe(OH) ₂ ⁺	Fe(OH) ₃ ⁰
0.5	-	-	-	0.029	-	-
1.0	-	-	-	0.065	-	-
2.0	0.0005	-	-	0.65	0.019	-
2.5	0.0016	-	-	2.04	0.19	-
3.0	0.005	-	-	6.46	1.85	-
3.5	0.016	0.0045	-	20.4	18.5	0.002

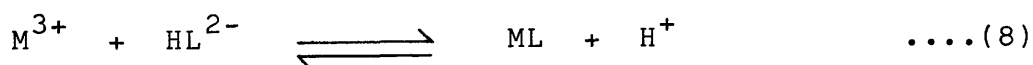
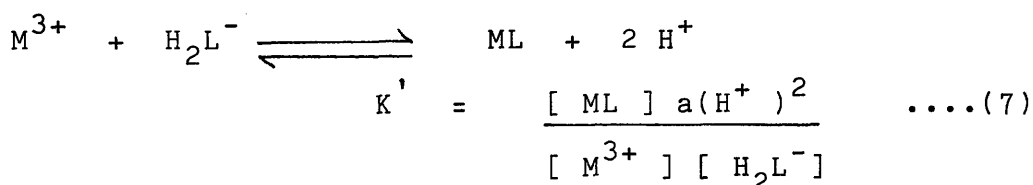
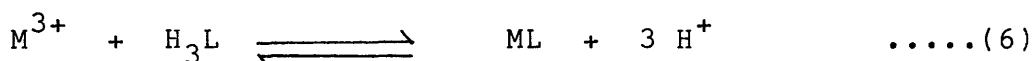
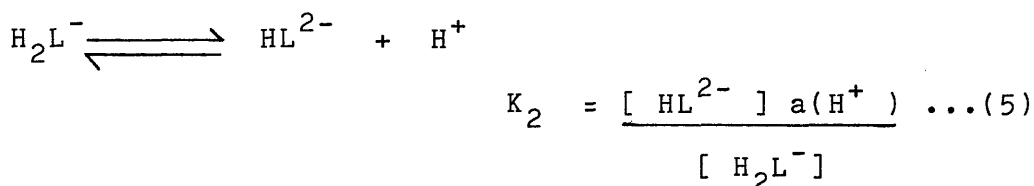
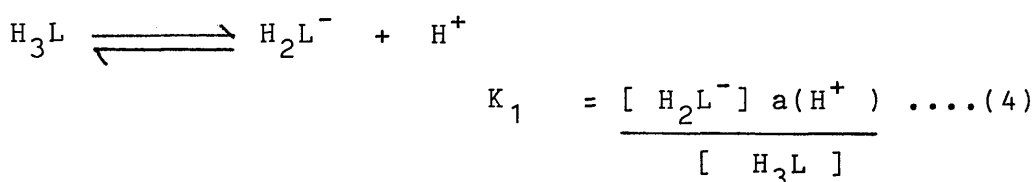
Glyphosate (H₃L) dissociates depending on the pH of the medium as below (Wauchope,1976) :



In the homogeneous system i.e. before formation of aluminium(111) and or iron(111) hydroxides and it is still acidic (pH < 7), the following species exist in the solution :

H_3L , H_2L^- , HL^{2-} , H^+ , M^{3+} , $M(OH)^{2+}$, $M(OH)_2^+$ and $M(OH)_3^0$

and the following reactions occur :



where ML = complex of M^{3+} and glyphosate at 1:1 ratio.

The total concentration of metal (M) and ligand (L) are given by :

$$C_M = [M^{3+}] + [ML] + [M(OH)^{2+}] + [M(OH)_2^+] + [M(OH)_3^0] \dots\dots\dots(9)$$

$$C_L = [H_3L] + [H_2L^-] + [HL^{2-}] + [ML] \dots\dots\dots(10).$$

The above reactions and equations, especially (9) and (10), are valid with the assumption that the reaction or complex formation between hydrolysed metal ion species and glyphosate do not occur or are relatively negligible. Complex formation between hydrolysed metal ions species and glyphosate has not been reported either by Madsen et al., (1978) or Motekaitis and Martell (1985).

Experimental conditions to eliminate the occurrence of metal ion hydrolysis.

The experimental conditions can be simplified so that the hydrolysis process of trivalent metal ions is eliminated, for example at $\text{pH} < 3.5$ for aluminium(111). By referring to table 5.1 and the properties of glyphosate in the acidic aqueous solution, the reactions (1) - (3), (4) and (8) do not occur and the equations (9) and (10) become :

$$C_M = [M^{3+}] + [ML] \quad \text{.....(11)}$$

$$C_L = [H_3L] + [H_2L^-] + [ML] \quad \text{.....(12)}$$

As in all experiments, concentration of metal ion was equal to concentration of glyphosate ($C_M = C_L$). So from the equations (11) and (12)

$$[M^{3+}] = [H_3L] + [H_2L^-] \dots\dots(13)$$

put equation (4) into (13) :

$$[M^{3+}] = [H_2L^-] \frac{a(H^+)}{K_1} + [H_2L^-]$$

$$[M^{3+}] = [H_2L^-] \left[1 + \frac{a(H^+)}{K_1} \right] \dots\dots(14)$$

where $a(H^+)$ is defined as 10^{-pH} .

A further equation is the proton balance equation, which yields,

$$B + [H^+] - [OH^-] = [H_2L^-] + 2.5 [ML] \dots\dots(15)$$

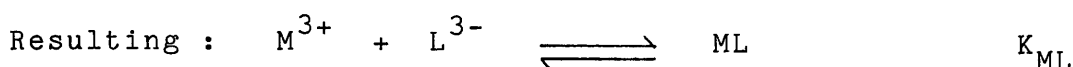
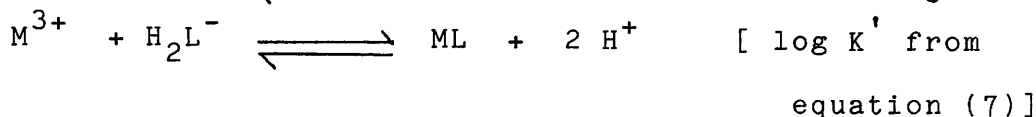
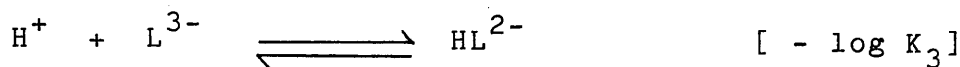
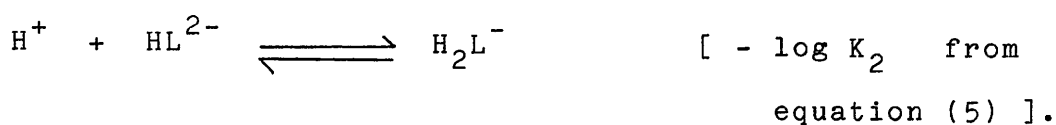
From equations (4) - (15) above, there is no $[L^{3-}]$ species existing in the solution to enable us to directly calculate the complex stability constant of trivalent metal ions and glyphosate at 1:1 ratio as follows :



and

$$K_{ML} = \frac{[ML]}{[M^{3+}][L^{3-}]}$$

So an indirect approach had to be used instead, with the following steps :



and

$$\log K_{\text{ML}} = - \log K_2 - \log K_3 + \log K' \quad \dots (17)$$

From equation (16), the complex stability constant of trivalent metal ion - glyphosate could be calculated if K' of equation (7) was known, because the values of K_2 and K_3 were obtained from the results of experiments of titration curves without metal.

Steps to obtain the value of K' :

equation (11) x 2.5 - (15).

$$2.5 C_M - (B + [\text{H}^+] - [\text{OH}^-]) = 2.5 [\text{M}^{3+}] - [\text{H}_2\text{L}^-] \quad \dots (17)$$

By using equation (14) and (17), $[\text{M}^{3+}]$ and $[\text{H}_2\text{L}^-]$ could be found.

From equation (12), $[\text{ML}]$ could be obtained. Therefore the K' value could be calculated from equation (7).

Calculations were done on Microtab on BBC Microcomputer, otherwise it would be very time consuming.

5.3 RESULTS AND DISCUSSION.

Five titration curves were recorded for free ligand (glyphosate) and four for aluminium - glyphosate complex. Titration curves for the iron(111) - glyphosate system could not be recorded because a precipitate formed throughout the experimental conditions. The calibration pH was rechecked after each run of a titration curve and no deviation exceeded 0.005 pH units.

The shapes of the curves where the pHs of glyphosate alone and glyphosate - aluminium complex were plotted against the volume of 0.05 M NaOH added (figure 5.2) and a (Mole of NaOH added/Mole of glyphosate present) (figure 5.3). It was found that for the aluminium - glyphosate complex system, a precipitate of aluminium hydroxide formed at pH ca. 4.3 or a about 2.8. As aluminium hydroxide is an amphoteric hydroxide (Vogel,1964), it was quite interesting to see the system of aluminium - glyphosate complex with increasing hydroxide ion. This graph is presented in figure 5.4.

The relationship between the pH and addition of NaOH to the iron(111) - glyphosate system could not be obtained because a precipitate formed throughout the experimental pH range.

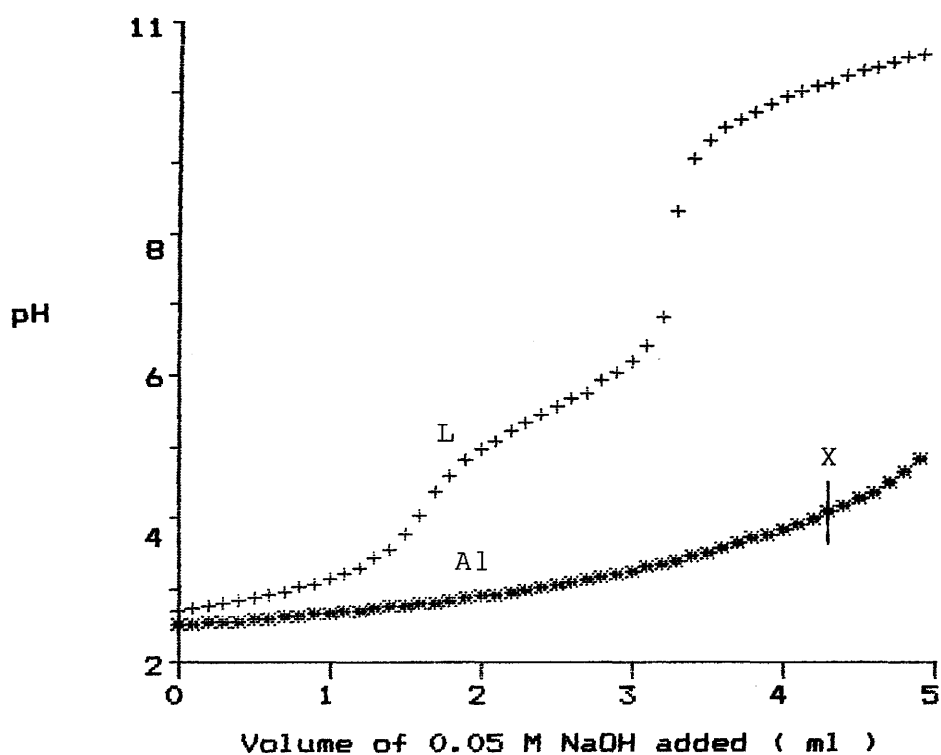


Figure 5.2 Graph of pH against volume of NaOH added
 + - glyphosate alone
 * - glyphosate + aluminium (III)
 $I = 0.10 \text{ (NaClO}_4 \text{)}$

On the Al curve X indicates the pH at which an insoluble phase formed.

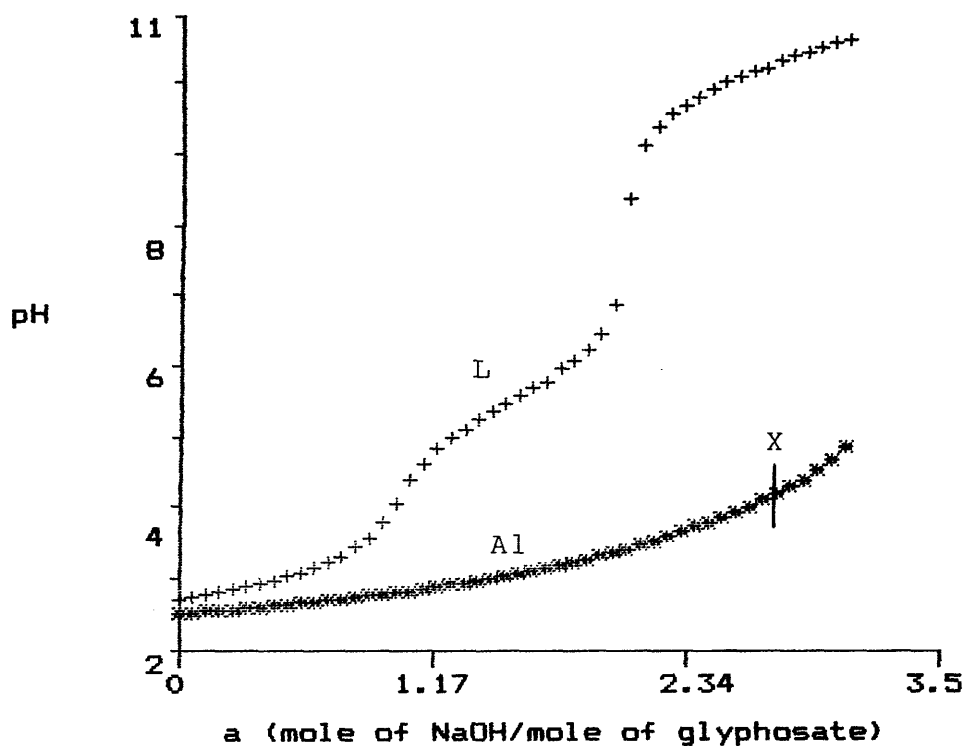


Figure 5.3 Graph of pH against mole of NaOH/mole of glyphosate.

+ - glyphosate alone

* - glyphosate + aluminium (111)

$I = 0.10$ (NaClO_4)

On the Al curve X indicates the pH at which an insoluble phase formed.

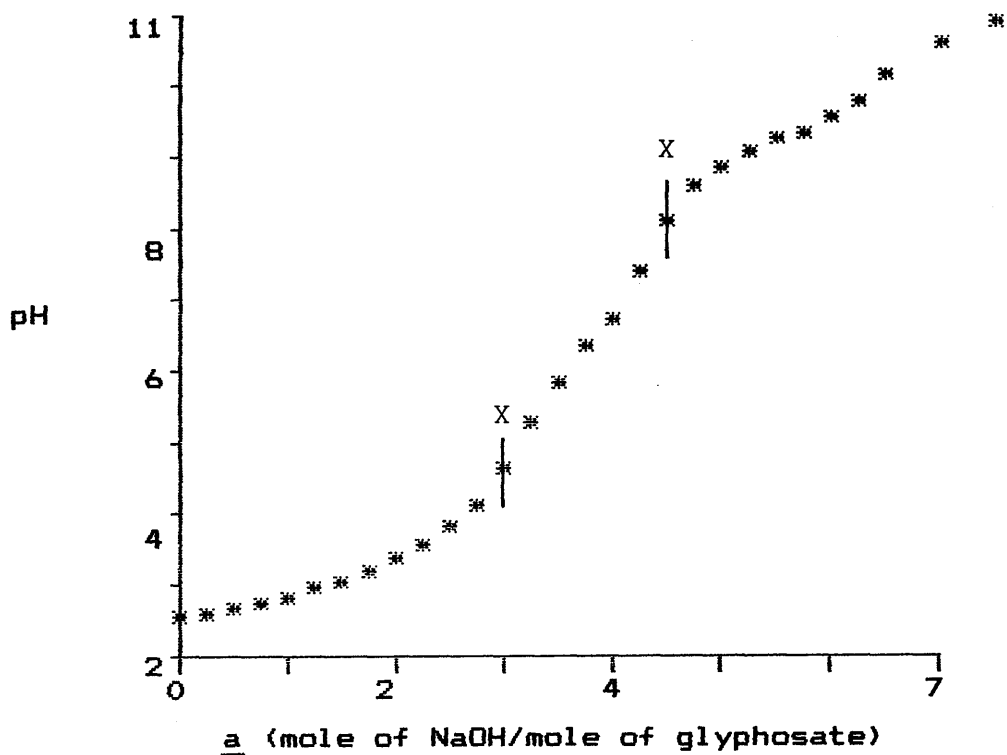


Figure 5.4 Graph of pH against mole of NaOH/mole of glyphosate for Al(III)-complex.

$I = 0.10 \text{ (NaClO}_4 \text{)}$

X - indicates the pH at which an insoluble phase formed.

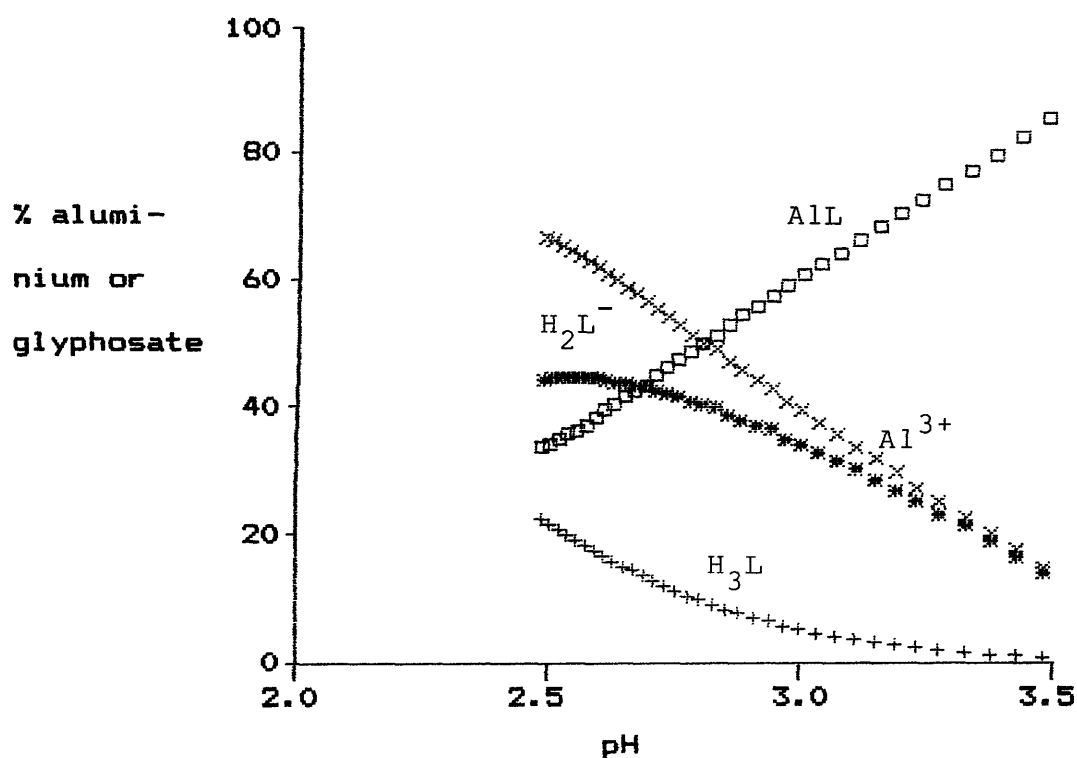


Figure 5.5 Distribution of species in aluminium -
glyphosate system at different pH
(% of total aluminium or glyphosate).
 $I = 0.10$ (NaClO_4)

* - $[\text{H}_2\text{L}^-]$

+ - $[\text{H}_3\text{L}]$

x - $[\text{Al}^{3+}]$

□ - $[\text{AlL}]$

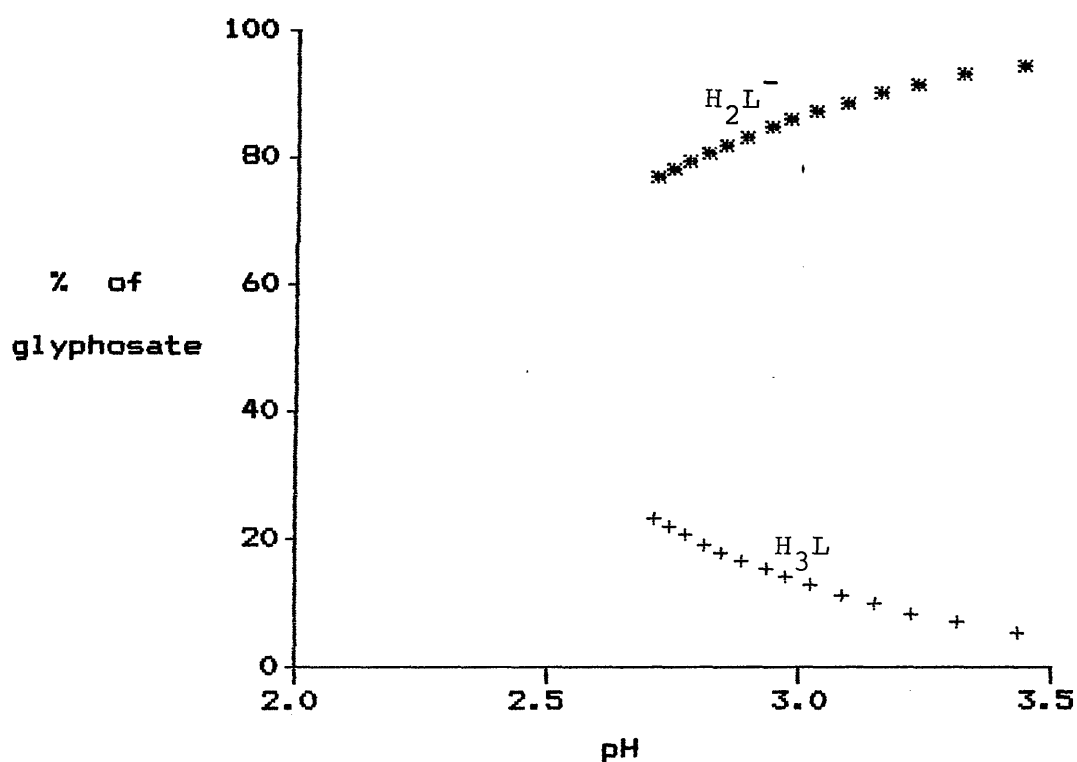


Figure 5.6 Distribution of species in glyphosate solution at different pH.

$I = 0.10$ ($NaClO_4$)

+ - $[H_3L]$

* - $[H_2L^-]$

Ligand.

The calculated dissociation constants for glyphosate are given in table 5.2.

Table 5.2 : Dissociation constants of glyphosate at 25°C
in I = 0.10 (NaClO₄).

Experiment No.	pK ₁	pK ₂	pK ₃
1	2.19	5.45	10.17
2	2.16	5.47	10.15
3	2.20	5.43	10.17
4	2.18	5.40	10.16
5	2.20	5.41	10.11
Mean	2.19 ± 0.02 ^a	5.43 ± 0.03	10.15 ± 0.02

a = ± standard deviation about mean.

From table 5.2, pK values for dissociation constants or log protonation constants if compared to the reported work in the literature (Wauchope, 1976; Madsen et al., 1978; Motekaitis and Martell, 1985) are all ca. 0.1 log units lower. The difference was most probably due to the different media used in the experiments.

Starting with totally deprotonated glyphosate, the successive protonation sites are, in order, the amino nitrogen, the phosphonate oxygen and finally the carboxylate oxygen atom. Thus the natural form of

glyphosate (H_3L) is a dipolar ion possessing a positively charged protonated amino group and a negatively charged hydrogen phosphonate group. The relative basicities of the various donor groups will be of some importance in assigning the bonding sites for chelate formation between the ligand and metal ion in the protonated chelates formed.

5.3.1 1:1 Aluminium chelate of glyphosate.

The chelate of aluminium is very stable as shown in figures 5.2 or 5.3. This can be inferred from the quite large depression that this trivalent metal ion produced in the corresponding pH equilibrium curves compared to glyphosate alone.

Although from figures 5.2 or 5.3 there was no inflection from the titration curve before formation of aluminium hydroxide to show that the aluminium - complex was formed, it had been reported that (Motekaitis and Martell, 1985) this complex did form. The results obtained here supported this, although there was a relatively low value for K' and high value for K_{AlL} . The low value of K' was due to this complex occurring and calculated in a low pH medium.

The calculated complex stability constant for aluminium - glyphosate at $25^{\circ}C$ in $I = 0.10$ ($NaClO_4$) is presented in table 5.3.

Table 5.3 Log stability constant of Al - glyphosate complex at 25 °C in I = 0.10 (NaClO₄).

Experiment No.	$K' = \frac{[AlL]}{[Al^{3+}][H_2L^-]} a(H^+)^2$	$K_{AlL} = \frac{[AlL]}{[Al^{3+}][L^{3-}]}$
1	- 2.50	13.08
2	- 2.55	13.03
3	- 2.53	13.05
4	- 2.57	13.07
Mean	- 2.54 ± 0.03 ^a	13.04 ± 0.03

a = ± standard deviation about the mean.

Since there was no totally deprotonated glyphosate (L³⁻) existing in the solution as mentioned earlier to form an aluminium - glyphosate complex because of its high complex stability constant, therefore reaction between aluminium ion and glyphosate shifted towards this complex formation, as in reactions (6) - (8).

There is a possibility of formation of AlHL⁺, protonation of AlL. However Motekaitis and Martell (1985) indicated that the formation of this complex was relatively low because of its low stability and relatively high pK for dissociation.

From figure 5.5, it is clear that the amount of aluminium-glyphosate complex increased with increasing pH of the medium and other species decreased sharply under

the same conditions. In the same conditions, without aluminium ion, the amount of H_2L^- increased with increasing pH (figure 5.6). This further supported the possibility of the formation of aluminium - glyphosate even under low pH conditions in which totally deprotonated glyphosate did not exist.

From table 5.3, the value of $\log K_{ML}$ for a 1:1 aluminium - glyphosate complex in perchlorate medium, $I = 0.10$ ($NaClO_4$) is 13.04. This value if compared to other work reported (Motekaitis and Martell, 1985), is ca. 0.6 log unit lower. The difference is most probably because of the different anionic medium used in both experiments. However the methods used here have the advantage of the simplicity of the calculations.

5.3.2 Iron(III) chelate of glyphosate.

The stability constant of iron(III) - glyphosate complex could not be determined in this experiment, i.e. in $I = 0.10$ ($NaClO_4$) because of formation of yellowish white precipitate throughout the experiment. When a lower concentration of glyphosate and iron(III) or only a lower concentration of iron(III) were used, a white precipitate was formed. The stability constant had been determined but in different medium, $I = 0.10$ ($NaNO_3$) (Motekaitis and Martell, 1985). In their experiment, in nitrate medium, Motekaitis and Martell (1985) also faced the same problem of a precipitate forming throughout the experiment in determining the complex stability constant

of lead(II) - glyphosate. They did not discuss this phenomenon.

Initial concentrations of iron(III) and glyphosate were 2.67×10^{-3} M and the pH of the mixture was about 2.2. Under the same experimental conditions, iron(III) perchlorate or glyphosate alone had a pH about 2.69 and 2.71 respectively, and there was no precipitate formed. From the above information it could be concluded that the precipitate formed was from the reaction of Fe^{3+} and glyphosate in perchlorate medium. This type of reaction which produced a precipitate did not happen in nitrate medium as mentioned earlier.

In order to understand more about the precipitate that forms from the iron(III) perchlorate - glyphosate reaction, the following experiments were carried out :

- i. Effect of concentration of iron(III) perchlorate and glyphosate.

Both the concentration of iron(III) perchlorate and glyphosate were lowered to 6.67×10^{-5} M. At this low concentration, the precipitate was still formed but in a lower amount. Therefore it could be said that the amount of precipitate formed would be less when concentrations of both chemicals were lowered. An attempt was also made to bring about a reaction at a 2:1 ratio of glyphosate to iron(III) because in nitrate medium, Motekaitis and Martell (1985) reported that this type of complex was also formed. Concentrations of glyphosate and iron(III) perchlorate used were 2.67×10^{-3} and 1.33×10^{-3} respectively.

The mixture of this 2:1 molar ratio also produced a precipitate (in perchlorate medium).

It was found that when the concentration of glyphosate was kept constant, the amount of precipitate formed increased as the concentration of iron(III) perchlorate increased. This phenomenon was also observed by Hensley et al., (1978) although in a different medium. They found that a precipitate was formed in solution containing FeCl_3 plus 1.04×10^{-4} M glyphosate. The amount of precipitate formed increased with increasing FeCl_3 concentration. A solution of FeCl_3 without glyphosate remained amber and no precipitate was formed. There was no colour change or precipitate when glyphosate and various concentrations of AlCl_3 were mixed.

ii. Effect of acidity or alkalinity.

The solubility of this precipitate was checked at lower and at higher pH. A titration curve at higher pH was observed by adding 0.2 M NaOH. The colour of the precipitate changed from a yellowish white (pH ca. 2.2) to yellow (pH ca. 2.5), then to brownish yellow (pH ca. 5.0), then an orange coloured solution was produced (without any precipitate at pH ca. 6) and finally a reddish brown precipitate formed again (ferric hydroxide at pH > 8). The reddish brown precipitate persisted at higher pH. This is more evidence indicating that the complex stability constant of iron(III)- glyphosate is very high and is higher than of aluminium - glyphosate because it could

prevent the formation of ferric hydroxide at a relatively high pH. This could be compared to a Fe(111) - EDTA complex where the EDTA prevents the formation of ferric hydroxide at $\text{pH} < 9$ (White, 1981). In nitrate medium, there was no precipitate observed up to pH ca. 6.8 (Motekaitis and Martell, 1985).

At lower pH, the precipitate could be dissolved either by adding HNO_3 , HCl or HClO_4 producing a colourless solution. It was found that this precipitate dissolved at pH ca. 0.6. To make the medium inert, HClO_4 was used to dissolve the precipitate. When the pH of the solution at which the precipitate dissolved was increased by adding 1 M NaOH, the precipitate formed again at pH ca. 1.2 (for 2:1 glyphosate to iron(111) ratio). When the amount of iron(111) present was increased, the precipitate started to form at lower pH (< 1.2).

Possible explanations of why mixtures of iron(111) and glyphosate formed precipitates in perchlorate medium were explored in this section as a preliminary study mainly based on the above simple experiment and from a literature survey.

From the above experiment and from Motekaitis and Martell (1985), it was clearly shown that there was an anion effect in the medium of the mixture of iron(111) and glyphosate. In nitrate medium there was no precipitate but in perchlorate medium a precipitate formed, although

in both conditions the ionic strength was the same ($I = 0.10$).

Ferric ions can form complexes with nitrate ions with a complex stability constant at 25 °C of 10 (Lindsay, 1979) but they cannot form complexes with perchlorate ions (Stumm and Morgan, 1981).

In a manner similar to that adopted in table 5.1, it could be calculated that at the initial experimental pH (ca. 2.2), the amount of hydrolysed species of iron(III) was slightly higher than Fe^{3+} especially $\text{Fe}(\text{OH})^{2+}$. $\text{Fe}(\text{OH})_2^+$ also existed but in a relatively low amount. At that pH, it could be that either Fe^{3+} or $\text{Fe}(\text{OH})^{2+}$ reacted with glyphosate and formed a precipitate or possibly both species were involved.

Since Fe^{3+} could form a complex with glyphosate and this complex was reported to be soluble (Motekaitis and Martell, 1985) and the complex formed was through the phosphonic group of glyphosate as it could not happen at the carboxylate group (Knuuttila and Knuuttila, 1985; Motekaitis and Martell, 1985), it could be said that the precipitate that formed in the mixture of iron(III) and glyphosate in perchlorate medium was not a complex of iron(III) - glyphosate. Since the carboxylate group of glyphosate was not involved in the formation of soluble iron(III) - glyphosate complex, it could be predicted that this carboxylate group and not hydroxide was most probably involved in the formation of the precipitated compound (the precipitate was white not reddish brown).

Under the initial experimental conditions (pH ca. 2.2) and from table 5.1 and figures 5.5 and 5.6, both species of iron(III) - Fe^{3+} and $\text{Fe}(\text{OH})^{2+}$ and both species of glyphosate - H_3L and H_2L^- existed in the solution. Also, since neutral glyphosate (H_3L) can only react with Fe^{3+} through a coordination reaction, this type of reaction can be neglected in the formation of the precipitated compound.

From the above experiment it was found that the precipitate dissolved at pH ca. 0.6 and reprecipitated again at pH ca. 1.2. This precipitate dissolved at pH > 6.

From the K_1 and K_2 values of glyphosate and α_1 , α_2 , α_3 values for hydrolysed species of Fe^{3+} , the existing species under various conditions can be calculated as in table 5.4.

Table 5.4 Fraction (%) of different species existing at different pH.

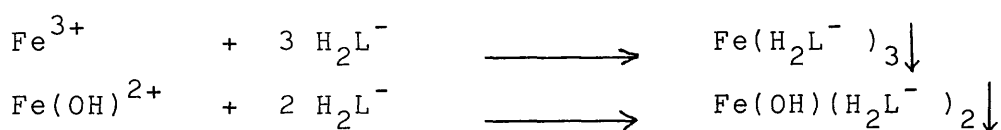
pH	H_3L	H_2L^-	HL^{2-}	Fe^{3+}	$\text{Fe}(\text{OH})^{2+}$	$\text{Fe}(\text{OH})_2^+$	$\text{Fe}(\text{OH})_3^0$	
0.6	98	2	-	97.4	2.6	-	-	**
1.2	91	9	-	89.8	10.2	-	-	***
5	-	83	27	0.005	3.1	96.7	0.38	*
6	-	21	79	0.00005	0.3	95.7	3.8	**

* - precipitate existed.

** - precipitate dissolved

*** - reprecipitate formed.

From table 5.4, it could be seen that the precipitate dissolved when the amount of H_2L^- species was decreased or amount of H_3L was increased (at low pH). The precipitate still existed in the solution when there was no H_3L species in the solution (pH = 5). This further supported the idea that species of H_2L^- not H_3L were involved in the formation of the precipitated compound. However it is still not clear whether it is the reaction of H_2L^- with Fe^{3+} or with $Fe(OH)^{2+}$ that formed the precipitate as depicted in the reaction below :

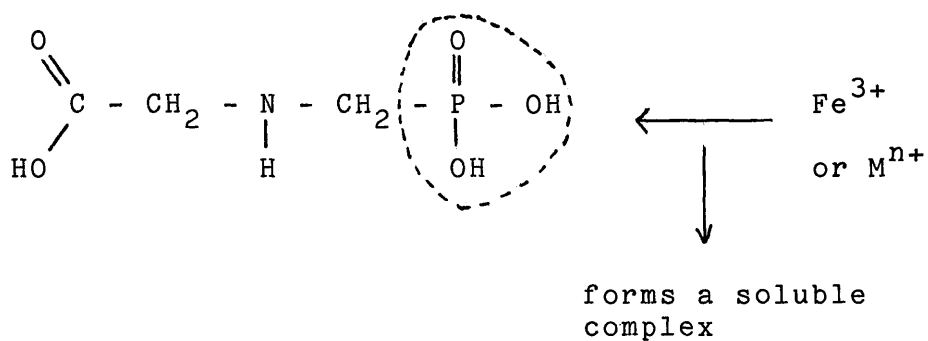


By comparing the solubility of the precipitated compound and % of H_2L^- , Fe^{3+} and $Fe(OH)^{2+}$ species existing at different pHs, from table 5.4 it is probable that the reaction between Fe^{3+} and H_2L^- forms the precipitate.

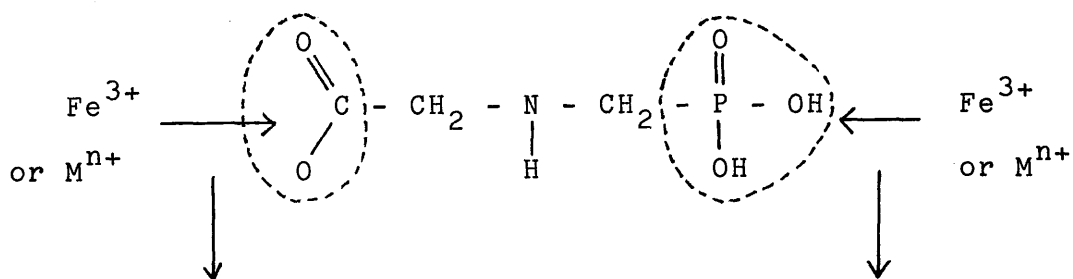
Based on the above information the reaction between glyphosate and iron(III) at low pH can be tentatively presented as in figure 5.7.

Figure 5.7 Preliminary proposal for the reaction between iron(III) and glyphosate.

H_3L species (pH < 1.2).



H_2L^- species (pH > 1.2).



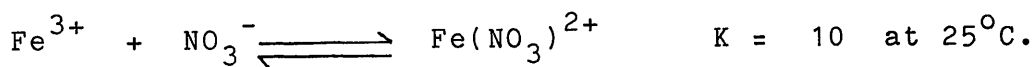
forms a precipitate
like ordinary acid-
base reaction that
forms a salt

forms a soluble
complex

From figure 5.7, it could be seen that glyphosate reacted with metal ions at both functional groups. At the phosphonate group it formed a soluble complex but at the carboxylate group it formed a salt where its K_{sp} would be different with different metal ions. In the case of iron(III) it could be postulated that its K_{sp} value was extremely low. It could be predicted that the K_{sp} value of aluminium - H_2L^- was higher than that of iron(III) because under the same conditions, no precipitate formed.

The above approach could be used to explain why in nitrate medium iron(III) - glyphosate mixture did not form any precipitate whereas in perchlorate medium a precipitate was formed with this mixture. The explanation is probably as follows :

ClO_4^- does not form a complex with Fe^{3+} but NO_3^- does.



where

$$K = \frac{[Fe(NO_3)^{2+}]}{[Fe^{3+}][NO_3^-]} \quad \dots\dots\dots(18)$$

in experiment of I = 0.10 ($NaNO_3$), $[NO_3^-] = 0.1$, and equation (18) becomes :

$$\begin{aligned} [Fe(NO_3)^{2+}] &= 10 \times (0.1) [Fe^{3+}] \\ &= [Fe^{3+}] \quad \dots\dots\dots(19). \end{aligned}$$

From equation (19) it can be deduced that the amount of free Fe^{3+} was reduced in nitrate medium more than in perchlorate medium.

Under the experimental pH conditions, this lower amount of Fe^{3+} also undergoes hydrolysis as in table 5.1 where its amount was reduced. The amount of free Fe^{3+} was further lowered because it also formed a complex with glyphosate. When the amount of free Fe^{3+} fell to a low concentration, the concentration of $\text{Fe}^{3+} - \text{H}_2\text{L}^-$ did not exceed its K_{sp} value, so a precipitate did not form. This is one of the possibilities explaining why the iron(111) - glyphosate system did not form a precipitate in nitrate medium but a precipitate formed in perchlorate medium. This was further supported from this work which showed that the amount of precipitate formed would reduce when less iron(111) perchlorate was added.

This approach could also be applied in explaining why Hensley et al., (1978) found that a precipitate was formed when the solution of glyphosate was mixed with FeCl_3 solution and the amount of precipitate formed increased with the increase in the concentration of FeCl_3 . In that study the ionic strength of the anionic medium (chloride ion) was relatively low.

5.4 ENVIRONMENTAL IMPLICATIONS.

In this section, environmental implications of glyphosate are discussed according to its chemical behaviour especially related to the above experiments. Although glyphosate is known to be degraded by microorganisms (Nomura and Hilton, 1977; Rueppel et al., 1977; Sprankle et al., 1975b) and thus influence its environmental behaviour, this aspect is not discussed in this section.

The complex stability constant of aluminium - glyphosate at a 1:1 ratio is $K = 1.1 \times 10^{13}$ in perchlorate medium. The complex stability constant of iron(III) - glyphosate could not be determined in perchlorate medium but its value in a nitrate medium is $K = 1.23 \times 10^{16}$. From this value it is found that the affinity of glyphosate with iron(III) is stronger than that with aluminium. However, in a relatively inert medium (perchlorate) or in low ionic strength media as had been studied by Hensley et al., (1978), it could form a complex and precipitate with iron(III). Besides forming 1:1 complex (ML type) it also could form 2:1 complex (ML_2^{3-} type) (Motekaitis and Martell, 1985) where the 2:1 complex stability constant was higher than the 1:1.

Compared with other metal ions, the affinity of glyphosate with aluminium and iron(III) are both strong (Madsen et al., 1978; Motekaitis and Martell, 1985). These aluminium and iron(III) - glyphosate complexes or precipitates were found to be relatively non toxic

compared to glyphosate itself (Hensley et al., 1978; Sprankle et al., 1975; Will et al., 1985). So from this information it could be predicted that iron(III) and aluminium will be the best metal ions to reduce the toxicity of glyphosate in the environment. This is further supported by earlier work where they found that the soils containing large quantities of iron(III) and aluminium adsorbed the greatest amount of glyphosate and thus reduced the phytotoxicity of glyphosate (Glass, 1987; McConnell and Hossner, 1985; Sprankle et al., 1975).

The formation of glyphosate complexes with aluminium is an equilibrium and reversible process. In the allophane test, the fluoride ion is used to chelate with aluminium thereby releasing alkalinity because in solution, fluoride complexes with aluminium at least as strongly as hydroxyl ions (Wild, 1988). It could be predicted that the same thing would happen if glyphosate falls on a soil containing allophane because the complex stability constants of glyphosate - aluminium either at 1:1 and or 2:1 are higher than 1:1, 2:1, 3:1, and 4:1 of the fluoride aluminium complexes (Lindsay, 1979; Motekaitis and Martell, 1985). Glyphosate will be complexed by aluminium and will release hydroxide into the environment.

When dealing with trivalent metal ions such as aluminium and iron(III), availability of active species of these ions could affect the formation of these ion - glyphosate complexes. These metal ions are insoluble and form hydroxides even under acidic conditions ($\text{pH} < 7$) because their hydroxides K_{sp} values are extremely low

(Ringbom, 1963). From figure 5.2 it can be seen that the aluminium- glyphosate complex was not stable at pH > 4 where precipitate of aluminium hydroxide formed by the following reaction :



The above reaction occurred even under acidic conditions (pH ca. 4) because $\log K_{sp}$ of aluminium hydroxide at 25°C is - 33.7 (Hogfeld, 1982). From the reaction it can be seen that glyphosate is released from the complex at higher pH and may again become an environmental problem. At low pH less environmental problems could be envisaged because of the complex formation or precipitate. This supported what had been obtained by McConnell and Hossner (1985) when they found that the adsorption of glyphosate to nontronite saturated with Al^{3+} decreased with increasing pH from 2.0 to 7.0.

The same effect could happen to iron(III) - glyphosate complexes. Although glyphosate can form a strong complex and a precipitate with iron(III), thus reducing its toxicity, the experiment carried out here showed that this precipitate dissolved again at pH ca. 6 - 8 and a precipitate of iron(III) hydroxide formed at pH > 8. From this observation it could be postulated that this iron(III) - glyphosate complex and precipitate that reduced the phytotoxicity of glyphosate at low pH is unstable at higher pH and released free glyphosate to the environment.

Glass (1987) had found that in acidic conditions, where glyphosate was equilibrated with Cu^{2+} - montmorillonite, a Cu - glyphosate complex was detected in the solution. Cu ions were brought into solution via a cation exchange action with solution protons and formed a complex with glyphosate. With this information and coupled with earlier and recent (Miles and Moye, 1988) findings, it can be proposed that in soil solution especially at low pH, glyphosate can form a complex with free Cu ions in the solution as well as with the metal ions bound to inter-layer spaces of the clay minerals. This mechanism could also happen to aluminium and iron(III) in soils where these free metal ions exist in low pH solution and their complex stability constants to glyphosate are higher than for Cu^{2+} .

Glyphosate could form complexes at 1:1 and 2:1 with metal ions that bind to clay and with free metal ions in the solution. Due to its higher complex stability constant in solution, Fe^{3+} could adsorb higher amounts of glyphosate compared with Cu^{2+} and these complexes were soluble. These soluble complexes could exist together with unadsorbed glyphosate and be mistaken for unadsorbed glyphosate. In the determination of adsorbed glyphosate in a clay (e.g. McConnell and Hossner, 1985; Glass, 1987), the amount of glyphosate in the solution was determined and subtracted from the amount added originally. The difference was taken as adsorbed. This complex is most probably mistaken as adsorbed glyphosate, consequently in

the calculation it reduced the amount of adsorbed glyphosate.

Another characteristic of trivalent metal ions such as aluminium and iron(III) in water is that they are easily hydrolysed even under acidic conditions, especially iron(III). From table 5.1 it can be seen that free Fe^{3+} is lower than its hydrolysed species at pH ca. 2.5 or higher. This effect is not so great for aluminium ions. When the unhydrolysed metal ions exist in lower amount (at higher pH), so the metal ion - glyphosate complex formed is also less than at a lower pH. Consequently, inactivation of glyphosate by trivalent metal ions especially iron(III) will be less effective. This could explain why with bentonite clay saturated with Fe^{3+} and Al^{3+} , glyphosate was adsorbed more by Al^{3+} than Fe^{3+} (Sprankle et al., 1975). This is because the adsorption experiment for Fe^{3+} was done at pH 5.4 and for Al^{3+} at pH 4.2.

From table 5.1, at these pH values the amount of Fe^{3+} present was relatively low compared to Al^{3+} , so the adsorption capacity of the clay saturated with Fe^{3+} was lower than that saturated with Al^{3+} and as a result the adsorption by clay - Fe^{3+} was lower than by clay - Al^{3+} , although in theory clay - Fe^{3+} could adsorb more glyphosate than clay - Al^{3+} because the complex stability constant of glyphosate for Fe^{3+} is higher than for Al^{3+} .

Glyphosate could be adsorbed by soil with high organic matter and C.E.C but these two factors appeared to have less influence on the adsorption (inactivation)

process of glyphosate compared to aluminium and iron content of the soil (at low pH). Aluminium and iron(III) contents in the soil were considered as important constituents in reducing the glyphosate environmental problem because binding did not seem to be related to the cation exchange capacity of clay but rather to cations on the clay (Hensley et al., 1978). In soil under arable conditions (pH ca. 6), organic matter and C.E.C will be more important in the deactivation of glyphosate.

Glyphosate could enter the aquatic environment by direct application, in the case of use in or near water or by run off or leaching from land use. Phytotoxicity of glyphosate was reduced when hard water was used as carrier solution (Shea and Tupy, 1984) or when polyvalent cations increased in the carrier water (Carlson and Burnside, 1984).

Although glyphosate would be expected to be primarily deactivated in the aquatic environment by adsorption onto clay and silt sediments followed by biological break-down, this does not seem to occur readily in most flowing water systems (Tooby, 1985). Bowmer et al., (1986) with their experiment in river and drainage waters found only a minor proportion of glyphosate was adsorbed onto suspended solids, even in turbid irrigation water. In bioassay experiments, they found that there were no significant differences in using different sources of water, including distilled water.

Other factors which could influence glyphosate deactivation in the ordinary aquatic environment (mineral-bearing water) are aluminium and iron(III) which seldom exist as their free ions due to the pH of the aquatic medium which is normally above the formation of their hydroxides (Stumm and Morgan, 1981).

The role of aluminium and iron(III) in the deactivation of glyphosate in the aquatic environment would be expected to be of little significance but it would be interesting to speculate on their role at low pH because of above reason.

CHAPTER 6.

CONCLUSIONS.

The role of this chapter is to draw together an appreciation of the glyphosate situation at the end of the studies. Since there is not enough time to continue the study of many of the aspects covered, some suggestions for further investigations are also made.

The main aim of this thesis was to improve the existing method of determination of glyphosate residues in food crops because these types of crops are the most likely to be exposed to the herbicide glyphosate. The improvement was emphasised with regard to the clean-up procedure, so that the method of determination would also be improved, or at least by providing an alternative clean-up procedure, the analyst would have a wider choice for the analysis.

Although the main task was to improve the clean-up procedure, by evaluating this procedure the behaviour of glyphosate could be better understood especially in food crops. The pH of food crops normally lies between 3 and 7 (Robinson, 1987) and in this condition glyphosate exists in neutral, mono or dianionic forms. Since glyphosate mostly exists as an anion, therefore other co-extractive anions have a great influence on glyphosate behaviour whether on cation or anion exchanger or on chelating resin. Another complicating problem that will have an

influence in the analysis of glyphosate residue in food crops and environmental matrices e.g. soil is its complexing properties. It can form complexes with bivalent and trivalent metal ions that are present in the matrices. It was found that 2:1 (ML_2 type) complexes are more stable than 1:1 (ML type). The charges in the glyphosate molecule and glyphosate-complex compounds are not greatly different because either in the free glyphosate molecule or glyphosate-metal complex compounds the charges are negative. More negative charges exist in a 2:1 complex.

In terms of sensitivity the results obtained in this study did not really give a big improvement compared to some of the existing methods. However, in terms of simplicity of the method, using a simpler pre-column derivatization procedure compared to existing post-column derivatization (see table 1.2), there was an advantage. The clean-up procedure using activated carbon column chromatography as a clean-up technique was a new procedure and could act as an alternative procedure for the analyst compared to conventional cation and anion exchange resins.

In chapter 2, the behaviour of glyphosate alone and under the influence of co-extractives was demonstrated. Determination of glyphosate by GC was rather difficult to perform compared to HPLC. This was because in GC, all three functional groups should be derivatized to enable it to be retained by the GC column and then detected by EC detector. Due to the nature of its functional groups, single step derivatization was difficult and produced a

derivatized compound with a relatively high polarity, to enable it to be retained quite strongly on the available GC packing materials (Chrompack, 1986) and, the interference of impurities could not be manipulated or reduced. The determination of glyphosate residues by GC-ECD was still a difficult task (Moye and Deyrup, 1984; Deyrup et al., 1985). This thesis lends support to this remark.

Due to the nature of glyphosate, being an ionic and water soluble compound, analysis by HPLC is advantageous over GC. This is why the number of analytical methods for glyphosate residues developed for HPLC is far greater than for GC (Chemical Abstracts, 1977-1988). In the determination by HPLC, again due to the nature of glyphosate and the properties of its co-extractives, determination by post-column derivatization was preferred by many investigators over pre-column treatments (table 1.2 in chapter 1) although it required more instrumentation and careful maintenance as well as analytical experience. In post-column derivatization, the HPLC column acted as a clean-up as well as a separation column to reduce the impurities and interferences during the detection.

In this thesis all the determinations of glyphosate were carried out by HPLC pre-column derivatization mainly because of its simplicity.

In determination by GC, all glyphosate functional groups were derivatized whereas in HPLC only one functional group (amino) was derivatized. So the

derivatization procedure for HPLC was much simpler compared to GC.

Based on its pK_1 , the ability of glyphosate (clean sample) to be retained by a cation exchange mechanism was negligible. It was supported by the experiments carried out here which showed that there was no glyphosate retained on a cation exchange Bond-Elut cartridge at pH ca. 2, even although cation exchange resin has been used in isolating glyphosate (table 1.2). It was retained on an anion exchange cartridge especially at pH 12 where all of its functional groups were fully deprotonated. However, glyphosate in potato extracts behaved very differently; none could be retained on an anion exchange cartridge under similar conditions to the cleaned form which was retained strongly. This very clearly showed the influence of co-extractives, especially the anions or compounds that formed anions at higher pH, on the glyphosate affinity to the anion exchanger. It was shown that the selectivity of the anions and the great amount present in the extract, compared to glyphosate, played an important role in limiting the affinity of glyphosate for the anion exchanger. Although there was a possibility of removing these competing anions it was not a practical solution because they have the same anionic charge as glyphosate.

The other reason why this bonded silica small cartridge could not retain glyphosate from potato extract was due to the relatively small amount of adsorbent present (100 - 500 mg) where its capacity was lower than

the high amount of competing anions present in the extract. It has been shown that glyphosate could be retained/isolated by using a large amount of anion exchange resin (PAM,1977; Seiber et al., 1984; Cowell et al.,1986; Tuinstra and Kienhuis,1987). This made it possible to dilute the sample extract to a concentration that could be coped with on the cartridge, but this approach was not further considered because it would dilute the amount of glyphosate and consequently reduce the detection sensitivity.

Small cartridges of bonded silica material proved to be ineffective for isolating glyphosate from potato extract. Due to this incapability, the reverse approach was used; the impurities of the potato extract were retained on the column and relatively clean glyphosate passed through without any retention and was collected for determination. It was found that activated carbon, a mixture of semi coarse and powder in the form of column chromatography could give a better clean-up for glyphosate in potato extracts than Bond-Elut cartridges. Due to the property of activated carbon, a better recovery was obtained at high pH at the expense of an increase in the influence of impurities. By using potassium carbonate solution (pH ca. 11) it was found that higher recoveries were obtained and although the impurities also increased, they did not interfere with the glyphosate peak in the HPLC detector. The use of potassium carbonate has an added advantage because the derivatization should be

performed at pH 9 - 11, so that no pH adjustment need be done prior to derivatization.

The results showed that activated carbon, a mixture of semi coarse and powder, produced a reasonably good recovery of glyphosate which was 53 % at the 0.1 mg/kg level and the limit of detection was 0.05 mg/kg tuber. A better recovery was obtained with higher amounts of glyphosate in the tubers. It was up to 92 % at the 10 mg/kg level. If this approach is compared to existing clean-up procedures it has the advantages as follows :

- i. Activated carbon is relatively easily available and cheaper compared to resin type materials.
- ii. This clean-up procedure could be used for pre-column derivatization procedures with the advantages over post-column derivatization as mentioned in chapter 2.
- iii. It required only a single step clean-up procedure. Other clean-up procedures require two or three steps.

Based on the above reasoning it can be said that, the clean-up procedure recommended here provides a reasonably good alternative for the determination of glyphosate residues in potato tubers.

The above procedure was used to determine glyphosate residues in chapter 3.

The effect of application of glyphosate to the potato plants and tubers at different rates, was discussed in detail in chapter 3. The results showed that physical

effects on the tubers such as reduction of sprout length, number of eyes open and loss of resistance to disease and rotting generally increased with increase of glyphosate application rate. Higher amounts of glyphosate applied gave more severe symptoms. Glyphosate residues in the tubers also increased with increasing rate of application. These observations showed that translocation of glyphosate did occur from the leaves to the tubers.

From the above parameters it was found that reduction of sprout length and number of eyes open could be used as an assessment for tuber injuries by glyphosate but obviously with less accuracy. Unfortunately, the rotting effect could not be used for assessment because of the difficulty of differentiating between normal disease levels and a rotting caused by glyphosate contamination. The best parameter to show that the tubers had been contaminated by this compound was by determining its residue level. Although both parameters - rotting effect and residue level, had a linear relationship with the treatment application rate only the residue level could be used with confidence for the assessment of tuber injuries due to glyphosate.

From the statistical point of view, there was no significant difference between untreated tubers and the tubers that had been treated with the lowest rate, 0.09 kg/ha glyphosate. All the parameters observed such as sprout length, number of eyes open, rotting effect and the residue level showed no significant difference between these two treatments. However their field growth

performance was very difference. For instance this study showed that, untreated tubers produced 96 % healthy plants and 4 % unhealthy plants whereas the treated tubers (with the lowest rate) only produced 32 % healthy plants, 40 % unhealthy and 28 % which did not grow at all. Their yields also showed a big difference where untreated tubers produced big (ware size) daughter tubers but that treated tubers produced only a few small (seed size) daughter tubers. Untreated tubers produced much higher yields.

These observations supported the findings by earlier investigators (Lutman and Richardson, 1978; Worthington, 1985). They found that the lack of injury symptoms to the tubers did not necessarily mean that they were not affected by the chemical as sometimes, apparently undamaged tubers failed to form healthy plants.

In the particular case of the residue levels there was a decided difference between the treatment mean for control and low level (0.03 and 0.14 mg/kg respectively). The lack of significance between these two residues can be accounted for mainly in terms of the fourth analytical value for the control which was 0.11 mg/kg whilst the remainder of the controls were less than 0.02 mg/kg (see table 3.6 (A)). An explanation for this high value is difficult to come by but would have to be accounted for in terms of slight contamination of some of the control plants in the field at the time of treatment. This is a particularly important aspect as the work here would suggest that a critical value for glyphosate in seed potatoes would be ca. 0.1 mg/kg. This does underline the

importance of trace contamination of seed potatoes stocks by low levels of glyphosate and the difficulties of determination.

Other tubers with higher treatment rates (0.36 and 0.72 kg/ha glyphosate) normally did not produce any plants (yield), all of them rotting before harvest.

Again in chapter 4, small cartridges of bonded-silica materials were tried to retain glyphosate from barley extracts because barley contains large quantities of carbohydrate and less anions compared to potato. It was found that the performance of anion exchange Bond-Elut cartridges in retaining glyphosate from barley extracts was better than for potato extracts. The amount of glyphosate retained by the cartridge increased from non-retained in potato to ca. 29 % in barley. However the improvement was not great enough to make use of this cartridge as a clean-up material in the determination of glyphosate residues in barley. This observation also showed that the amount of anions existing in barley extract still had a great influence on glyphosate retention by the cartridge.

The cation exchange Bond-Elut cartridge was also found not to be suitable as a preliminary clean-up step especially to reduce the amount of amino acids present in the extract because its capacity was lower compared to the amount of cations which existed in the barley extract.

Batch addition of charcoal was investigated as a clean-up method for barley extract but in contrast to its success in column chromatography described in chapter 2,

it adsorbed some glyphosate and at the same time resulted in reduced resolution of the glyphosate-derivative in HPLC and made the quantitation less accurate.

Employing a chelating resin column to retain glyphosate was found not to be too successful. It showed that quite high amounts of glyphosate were not retained by this resin. This was probably due to the influence of other naturally occurring chelating compounds existing in barley. Although their complex stability constants to Fe(III) were lower compared to glyphosate the amounts present were extremely high compared to spiked glyphosate (table 4.5) which had a big influence on the retention of glyphosate by the resin.

The influence of these chelating compounds was clearly shown when the pH of the resin and extract were increased to ca. 4. These chelating compounds were retained more strongly by the resin and the amount of glyphosate retained was very low. This is because the complexing power of chelating compounds is increased with increasing pH (Schwarzenbach and Flaschka, 1969).

Recovery experiments using this resin gave 52 % at 1 mg/kg and 42 % at 0.1 mg/kg level. Another reason for the poor recoveries was that glyphosate was found to be present in the blank. This was discovered during low level recovery studies (0.1 mg/kg). A relatively high amount of glyphosate in the blank reduced the amount of spiked glyphosate retained by the resin and thus reduced its recovery. Recovery could be improved by using more chelating resin in order to increase the amount of

glyphosate retained but this approach would lengthen the clean-up process.

Dealing with the analysis of glyphosate residue in a high carbohydrate matrix such as barley, another problem that had to be overcome was the formation of gel. This gel formed a viscous extract solution that could not be precipitated out even after centrifuging at 11,000 rpm. This viscous solution reduced the contact between the compound and the clean-up material, thus reducing its retention. This could be partly coped with by adding a volume of acetone.

By keeping strictly to the aim of this chapter, i.e. to find a good method of analysis for glyphosate residues in barley, this was not fully achieved. However it was partially achieved in that a reasonable recovery was obtained (ca. 50 %). Other achievements that were gained from this chapter were that the behaviour of glyphosate and influences of co-extractive compounds were better understood. This information could be used for future work with glyphosate especially at low residue levels.

One of the characteristic properties of glyphosate that had been applied in chapter 4, i.e. its complexing property was studied in further detail in chapter 5. Glyphosate could form a very stable complex with trivalent metal ions especially aluminium and iron(III). The reaction between iron(III) and glyphosate was of interest because it formed a soluble compound in nitrate medium but produced a precipitate in perchlorate (inert medium). The precipitate also formed when the ionic strength of the

medium was relatively low (Hensley et al.,1978). It did not happen to the aluminium - glyphosate mixture where no precipitate occurred in both media (nitrate and perchlorate). The complex stability constant of glyphosate-Fe(III) was found to be the highest of the metal ions tested. The precipitated compound formed from Fe(III)-glyphosate mixture in perchlorate could not be a glyphosate-Fe(III) complex because both the 1:1 and 2:1 complexes had been reported previously in nitrate and they were both soluble compounds (Motekaitis and Martell,1985). Therefore it could be predicted that besides the reaction of forming a glyphosate-Fe(III) complex, glyphosate also reacted with Fe(III) to form another compound that was insoluble. This would need to be confirmed by future work.

The most probable reaction forming this compound was between the iron(III) and H_2L^- (deprotonated carboxylic group) species of glyphosate because the precipitate dissolved at a pH where the amount of this species was relatively low. Other functional groups such as protonated carboxylic, and phosphonic groups only formed a soluble complex with Fe(III). From the above information it could be predicted that the deactivation of glyphosate by Fe(III) would be the greatest. This was also observed by earlier investigators (Sprankle et al,1975; Hensley et al.,1978; McConnell and Hossner,1985; Glass,1987).

Complex stability constants of glyphosate and metal ions such as iron(III), Al, Cu and Ni were high. It also formed complexes with other divalent metal ions where

their complex stability constants were lower but did not form with monovalent metal ions. Most of these elements exist in the environment (especially soil and water) and in plants. Due to the high complex stability constants of metal ions with glyphosate, it could be expected that glyphosate, once it entered the environment, would be deactivated (bound) by these elements if they were present. In an environment where most of the above elements would be present in lower amounts i.e. in sandy soil, glyphosate had quite a long residual herbicide activity compared with most other soils (Korol,1985; Delvin et al.,1986).

The complex stability constants of glyphosate with metal ions could be applied to answer and to predict the fate of this herbicide in the environment. Soils with high contents of minerals especially iron and aluminium can readily deactivate glyphosate in the environment. Environments that have little bi- and trivalent metal ions such as sandy soil and natural water (rivers) would not be able to reduce glyphosate environmental problems easily.

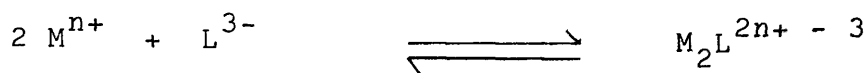
Deactivation of glyphosate especially in soil as mentioned above is mainly due to its complex formation with metal ions present in the soil. Due to the complex formation process, binding (deactivation) of glyphosate to the soil is not permanent (irreversible) because complex formation is an equilibrium and reversible process. Soil that has been contaminated with glyphosate even if it is showing no herbicide residual effect at the time, must be treated with caution. Care should be taken not to shift

the equilibrium process toward the re-releasing of glyphosate back to the environment and thereby creating an environmental problem. For example liming glyphosate contaminated soil would favour the formation of metal hydroxides and releasing glyphosate because the pK_{sp} of metal hydroxides are higher than their log complex stability constants with glyphosate.

Other aspects of glyphosate complexing characteristics which are largely ignored concern its implication in plants or crops especially related to its residue analysis. In residue analysis when the amount of glyphosate is low or when developing a method of residue analysis, glyphosate is spiked at relatively low concentration normally equivalent to 1 mg/kg or less, sometimes as low as 0.01 mg/kg. This is because the main objective of developing the new method of analysis is to increase the sensitivity of detection to as low a concentration as possible, particularly for as biologically active a compound as glyphosate.

In vegetables, fruits, cereals and other tuber crops the amounts of cations that could form complexes with glyphosate such as Ca, Mg, Fe, Cu and Zn are relatively high compared to the low level of spiked glyphosate (Briggs, 1978; Belitz and Grosch, 1987), especially when the amount of glyphosate was as low as 0.1 or 0.01 mg/kg. It was found that when the amount of metal was in excess as would be the case mentioned above, the formation of

bimetal and a ligand complex was dominant (Ringbom, 1963; Schwarzenbach and Flaschka, 1969).



Due to the fact that monovalent metal ions could not form complexes with glyphosate, M_2L type complexes are always positively charged - one positive charge with bimetal ions and three positive charges with trivalent metal ions. Because these types of complexes are positively charged, they can be retained on cation exchange resins. This phenomenon could explain why cation exchange resin was successfully used as a clean-up material to retain glyphosate in many residue analysis treatments (PAM, 1977; Moye and St. John, 1980; Guinivan et al., 1982; Roseboom, 1982; Moye et al., 1983; Archer, 1984). This possibility and its applications could be investigated further.

The remit from the grant awarding body was to gain experience in pesticide analysis.

This could have been accomplished along traditional lines by opting for multi-residue analysis of pesticides in a range of commodities.

In this case a deliberate attempt was made to select a problem which existed at present and was likely to increase in importance over the next few years. Hence glyphosate was chosen as it is a fast developing chemical, has proved difficult to estimate accurately in crops at

low levels, and is very phytotoxic. The fact that it is a substituted phosphate made it particularly interesting in terms of its solubility and complexing properties, and its similarity to existing chemicals present in plants.

Much effort in this work was given to developing a simple procedure for analysis where emphasis was given to clean-up treatments, both traditional and modern. By adopting this policy some success was gained in the development of simple analytical procedures for glyphosate. However, as with most research work of this type, many questions are raised as well as answered. Nevertheless, as well as new methodology some explanation for the separations achieved by others was derived from an understanding of the chemical properties of glyphosate itself and its co-extractives.

REFERENCES.

Allen, E.J.(1978). Plant density, in The Potato Crop (ed.P.M.Harris), Chapman & Hall, London.

Anon (1971). Salts and derivatives of MON 0573 post-emergence herbicide, Monsanto Agricultural Division Technical Bulletin 057-1-71.

Anon (1973). Progress report for the period April 1972 to March 1973. Institute of Agricultural Research, Melka Werer Research Station, Ethiopia : 157-164.

Archer,T.E. and Stokes,J.D.(1984). Residue analysis of glyphosate in blackberries by high performance liquid chromatography and post column reaction detection, J.Agric.Food Chem., 32 : 586-588.

Aveston,J.(1965). Hydrolysis of the aluminium ion : Ultracentrifugation and acidity measurements, J.Chem.Soc., 3 : 4438-4443.

Bardalaye,P.C., Wheeler,W.B. and Moye,H.A.(1985). Analytical techniques of glyphosate residue analysis in The Herbicide Glyphosate (eds. E.Grossbard and D.Atkinson), Butterworths, London : 263-285.

BDH catalogue (1987/88). Reagents, biochemicals, diagnostics, fine chemicals, Dorset. U.K.

Belitz,H.D. and Grosch,W.(1987). Food Chemistry,
translation from second German edition by D.Hadziyev,
Springer-Verlag Berlin, Heidelberg.

Bio-Rad Laboratory (1965). Materials for ion exchange,
adsorption, gel filtration, California. U.S.A.

Blau,K.and King,G.S.(1978). Handbook of derivatives for
chromatography, Heydon & Sons Ltd.,London.

Boehringer (1977/78). Methods of enzymatic food analysis,
Mannheim, West Germany.

Bowmer,K.H.,Boulton,P.M.D.,Short,D.L. and
Higgins,M.L.(1986). Glyphosate-sediment interactions and
phytotoxicity in turbid water, Pestic. Sci., 17 : 79-88.

Briggs,D.E.(1978). Barley, Chapman & Hall, London.

Bronstad,J.O. and Friestad,H.O.(1976). Method for
determination of glyphosate in natural waters based on
polarography of the N-nitroso derivative, Analyst, 101 :
820-824.

Bruner, F.(ed.) (1985). The science of chromatography,
Elsevier, Amsterdam.

Bruton, C.(1986). Fully automated amino acid analysis using pre-column derivatization, Int. Lab., June : 30-38.

Bunyatyan,Y.A. and Gevorgyan,A.A.(1984). Determination of the active ingredient of Round-up and its metabolite in environment, Git. Sanit : 43-44. Cited : Chem.Abs., 101-85018.

Burns,A.J. and Tomkins,D.F.(1979). The determination of N-(phosphonomethyl) glycine in formulation and technical samples by high pressure liquid chromatography, J. Chromatog. Sci., 17 : 333-335.

Burton,W.G.(1966). The potato : A survey of its history and of factors influencing its yield, nutritive value, quality and storage, 2nd edition, Veenman and Zonen Wageningen, Holland.

Calvet, R.(1980). Adsorption - desorption phenomena, in Interactions Between Herbicides and the Soil.(ed.R.J.Hance), Academic Press. London.

Carlson,K.L. and Burnside,O.C.(1984). Comparative phytotoxicity of glyphosate, SC-0224, SC-0545 and HOE-00661, Weed Sci., 32 : 841-844.

Chaberek, S. and Martell, A.E. (1952). Stability of metal chelates. IV. N-N'-ethylenediamine diacetic acid and N-N'-ethylenediamine diacetic-N-N'-dipropionic acid, J. Am. Chem. Soc., 74 : 6228-6231.

Chang, J.C. (1987). Solubility product constants, in CRC Handbook of Chemistry and Physics, 68th edition (ed. R.C. Weast), CRC Press, Florida : B-207-208.

Ching, S., Nomura, N., Yauger, W., Uyehara, G. and Hilton, N.W. (1976). Summary of glyphosate use, Hawaiian Sugar Planters Association 1975 Annual Report : 39-40.

Chrompack (1986). The chrompack guide to chromatography, general catalogue, Middelburg, Netherland.

Claus, J.S. and Behrens, S. (1976). Glyphosate translocation and quackgrass rhizome bud kill, Weed Sci., 24 : 149-152.

Colowick, S.P. and Kaplan, N.O. (eds.) (1955). Methods in Enzymology Vol. 1., Academic Press, New York.

Cooper, F.B. (1975). Herbicides for maize. Report on field experiments 1975. ADAS, E. Midlands Region MAFF, U.K. : 451-457.

Coupland,D. and Caseley,J.C.(1979). Presence of ^{14}C activity in root exudates and guttation fluid from Agropyron repens treated with ^{14}C - labelled glyphosate, New Phytol., 83 : 17-22.

Cowell,J.E.,Kunstman,J.L.,Nord,P.J.,Steinmetz,J.R. and Wilson,G.R.(1986). Validation of and analytical residue method for analysis of glyphosate and metabolite : An inter-laboratory study, J.Agric.Food Chem., 34 : 955-960.

Davidson,J.G.(1975). Glyphosate - a new weed killer for fruit growers?. ARC research review, 1 : 59-62.

Devlin,R.M.,Karczmarczyk,S.J.,Zbiec,I.I. and Koszanski,Z.K.(1986). Initial and residual activity of glyphosate and SC-0224 in a sandy soil, Crop Prot., 5 : 293-296.

Dewey,S.A. (1982). Manipulation of assimilate transport as a method of studying glyphosate translocation in tall morning-glory, Ph.D. thesis. Oregon State Univ. U.S.A. Cited : The Herbicide Glyphosate eds. E.Grossbard and D.Atkinson,1985 : 97.

Deyrup, C.L., Chang, S.M., Weintraub, R.A. and Moye, H.A. (1985). Simultaneous esterification and acylation of pesticide for analysis by gas chromatography. 1. derivatization of glyphosate and (aminomethyl)phosphonic acid with fluorinated alcohols-perfluorinated anhydrides, J. Agric. Food Chem., 33 : 944-947.

Dowdy, S. and Wearden, S. (1983). Statistics for Research, John Wiley & Sons, New York.

Dytyuk, L.T., Narozhnyi, G.A., Samakaev, R.K., Saltova, T.A., Zaripov, R.K. and Zaripov, N.R. (1982). Preventing mineral salts deposits from aqueous solutions, Prom. Obratsy, Tovarnye Znaki, 42 : 92. Cited : Chem. Abs., 98-132122.

Ekstrom, G. and Johansson. (1975). Determination of glyphosate (N-phosphono methyl glycine) using an amino acid analyzer, Bull. Environ. Contam. Toxicol., 14 : 295-296.

Elliot, J.G. and Wilson, B.J. (eds) (1983). The influence of weather on the efficiency and safety of pesticide application. The drift of herbicides, The British Crop Protection Council, London.

Environmental Protection Agency (1982b). Code of Federal Regulations : Protection of environment, 40 CFR : 180-364.

Fenot, P., Darriet, J., Garrigou-Lagrange, C. and Cassaigne, A. (1978). Crystallographic and vibrational studies of metal complexes of aminomethyl phosphonic acid, J. Mol. Struct., 43 : 43-60.

Food and Drug Administration (1980). Pesticide Analytical Manual, FDA, Washington.

Ford, D.L. (1981). Waste water characteristic and treatment, in Activated Carbon Adsorption For Waste Water Treatment (ed. J.R. Perrish), CRC Press, Florida.

Franz, J.E. (1974). N-phosphonomethyl glycine phytotoxicant composition. U.S. Patent 3,799,758, Monsanto Co.

Franz, J.E. (1985). Discovery, development and chemistry of glyphosate, in The Herbicide Glyphosate (eds. E. Grossbard and D. Atkinson), Butterworths, London : 3-17.

Friestad, H.O. (1978). Determination of glyphosate in water, soil and plant materials by means of differential pulse polarography, Nordic Plant Protection Conference 1978, Russilo, Finland : 83-85. Cited : The Herbicide Glyphosate eds. E. Grossbard and D. Atkinson, 1985 : 201.

Friestad, H.O. and Bronstad, J.O. (1985). Improved polarographic method for determination of glyphosate herbicide in crops, soil and water, J. Assoc. Off. Anal. Chem., 68 : 76-79.

Gitsova,S. and Bratano,Z.(1984). Determination of the residue of glyphosate and its metabolite in waters by thin layer chromatography, Khig. Zdraveopaz, 27 : 172-176.

Cited : Chem.Abs., 101-85522.

Glass, R.L.(1981). Colorimetric determination of glyphosate in water after oxidation to orthophosphate, Anal. Chem., 53 : 921-923.

Glass,R.L.(1983). Liquid chromatographic determination of glyphosate in fortified soil and water samples, J.Agric.Food Chem.,31 : 280-282.

Glass,R.L.(1987). Adsorption of glyphosate by soils and clay minerals, J.Agric.Food Chem., 35 : 497-500.

Glowiak,T.,Sawka-Debrowolska,W.,Jezowska-Trzebiatowska,B. and Antonow,A.(1980a). X-ray studies of cobaltate aminomethyl phosphonic acid polymeric complex, Inorg.Chim.Acta, 45 : L105-L106.

Glowiak,T.,Sawka-Debrowolska,W.,Jezowska-trzebiatowska,B. and Antonow,A.(1980b). Crystal and molecular structure of bis(aminomethyl phosphonate) copper(II), J.Cryst.Mol.Struct., 10 : 1-10.

Gomez,K.A. and Gomez,A.A.(1984). Statistical Procedures for Agricultural Research, 2nd edition, John Wiley & Sons, New York.

Gottrup,O.,O'Sullivan,P.A.,Schraa,R.H. and Vanden-Born,W.H.(1976). Uptake, translocation, metabolism and selectivity of glyphosate in Canada thistle and leafy spurge, Weed Res., 16 : 197-201.

Gougler,J.A. and Geiger,D.R.(1981). Uptake and distribution of N-phosphonomethyl glycine in sugar beet plants, Plant Physiol., 68 : 668-672.

Gray, D. (1973). The growth of individual tubers, Potato Res., 16 : 80-84.

Gray,D. and Smith,D.J.(1973). The pattern of assimilate movement in potato plants, Potato Res., 16 : 293-295.

Grossbard,E. and Atkinson,D.(eds.) (1985). The Herbicide Glyphosate, Butterworths, London.

Guinivan,R.A.,Thompson,N.P. and Wheeler,W.B.(1982). Derivatization and clean-up improvements in determination of residue of glyphosate and aminomethyl phosphonic acid in blueberries, J.Assoc.Off.Anal.Chem.,65 : 35-39.

Hance, R.J.(1976). Adsorption of glyphosate by soils, Pestic.Sci., 7 : 363-366.

Harris, P.M.(ed.) (1978). The Potato Crop, Chapman and hall, London.

Hassall, K.A.(1982). The Chemistry of Pesticides. MacMillan Press Ltd., London.

Hensley,D.L.,Beuerman,D.S.N. and Carpenter,P.L.(1978). The inactivation of glyphosate by various soil and metal salts, Weed Res., 18 : 287-291.

Hide,G.A. and Lapwood,D.H.(1978). Disease aspects of potato production, in The Potato Crop (ed.P.M.Harris), Chapman & Hall, London.

Hogfeldt, E.(1982). Stability Constants of Metal-Ion Complexes. Part A: Inorganic Ligands, Pergamon Press, Oxford, U.K.

Holly, K.(1985). Glyphosate : A summing up, in The Herbicide Glyphosate (eds.E.Grossbard and D.Atkinson), Butterworths, London.

Huber III,J.W. and Calabrese,K.L.(1985). Derivatization of glyphosate and alafosfalin for reverse-phase HPLC analysis, Liq.Chromatogr.HPLC Mag., 3 : 888-892.

Hutchins, R.A.(1981). Activated carbon, in Activated Carbon Adsorption for Waste Water Treatment (ed.J.R.Perrish), CRC Press, Florida, U.S.A.

Kader,M.H.M.A. and Ragab,M.T.H.(1977). Annual Report, Research Station, Kentville, Nova Scotia : 159-162.

Katz, E.(ed.) (1987). Quantitative Analysis using Chromatographic Techniques, John Wiley & Sons, New York.

Kent, N.L.(1984). Technology of Cereals, 3rd.edition, Pergamon Press, Oxford, U.K.

Kleifeld, Y. (1976). Control of Cynodon dactylon in cotton, Phytoparasitica, 4 : 148.

Korol, R.V.(1985). Setting standards for soil glyphosate content, Git.Sanit., 8 : 71-72. Cited : Chem.Abs., 103-136755.

Kragten, J. (1978). Atlas of Metal-Ligand Equilibria in Aqueous Solution, Ellis Horwood Ltd., Chichester, U.K.

Kubota, H. (1956). Properties of volumetric determination of aluminium ion, Dissertation, Univ. of Wisconsin, Diss.Abstr. 16, 864. Cited : The Hydrolysis of Cations, Baes,C.F. and Mesmer,R.F.,John Wiley & Sons,New York,1956.

- Laermann,H.T. and Lundehn,J.R.(1980). Weed control in lodge cereals - efficiency and residue situation, Nachrichtenbl.Dtsch. Pflanzenschutzdienst, 30 : 125-127.
- Lange,A.H. and Schlesselman,J.(1975). Tree and vine responses to glyphosate, Proceedings of the Western Society of Weed Science, 28 : 49.
- Laurie,I.S. and Wittwer,J.D.(eds.) (1983). High Performance Liquid Chromatography in Forensic Chemistry, Marcel Dekker, New York.
- Lawrence, J.F.(1981). Organic Trace Analysis by Liquid Chromatography, Academic Press, New York.
- Lindsay, W.L.(1979). Chemical Equilibria in Soils, John Wiley & Sons, New York.
- Liu,D.J.,Robbins.G.S. and Pomeranz,Y.(1974). Cereal Chem., 51 : 309-316.
- Liu,L.C. and Acevedo-Borrero,E.(1980). Chemical weed control in taniers, J.of Agric.of the Univ.of Puerto Rico, 64 : 442-449.
- Liu,L.C.,Rodriguez-Garcia.J. and Semidey-Laracuente,N. (1981). Glyphosate for weed control in plantains, J.of Agric.of the Univ.of Puerto Rico, 65 : 317-325.

Lolas,P.C. and Coble,H.D.(1980). Translocation of ^{14}C -glyphosate in Johnsongrass as affected by growth stage and rhizome length, Weed Res., 20 : 267-270.

Loomis,W.D. and Batteille.(1966). Plant phenolic compounds and the isolation of plant enzymes, Phytochem., 5 : 423-438.

Lovell,P.H. and Booth,A.(1967). Effect of gibberellic acid on growth, tuber formation and carbohydrate distribution in Solanum tuberosum, New Phytol., 66 : 525-537.

Lundgren, L.N.(1986). A new method for determination of glyphosate and (aminomethyl)phosphonic acid residues in soils, J.Agric.Food Chem., 34 : 535-538.

Lutman,P.J.W. and Richardson.W.G.(1978). The activity of glyphosate and aminotriazole against volunteer potato plants and their daughter tubers. Weed Res., 18 : 65-70.

Lutman,P.J.W.(1979). The effect of topical application of glyphosate and aminotriazole on volunteer potatoes (Solanum tuberosum), Weed Res., 19 : 377-383.

MacGregor,A.W. and Edwards,R.A.(1968). Ensilage of whole-crop barley. II. Composition of barley silage at different stages of growth, J.Sci.Food Agric., 19 : 661-666.

- Madsen, H.E.L., Christensen, H.H. and Petersen, C.G. (1978). Stability constants of copper(II), zinc, manganese(II), calcium and magnesium complexes of N-(phosphonomethyl) glycine (glyphosate), *Acta Chem.Scand.*, 32 : 79-83.
- Magambo, M.J.S. and Kilavuka, C.I. (1982). Effect of glyphosate on shoot growth of tea, *Trop.Pest Manage.*, 28 : 315-316.
- Martin, J.T. and Juniper, B.E. (1970). *The Cuticle of Plants*, Edward Arnold Ltd., London.
- McConnell, J.S. and Hossner, L.R. (1985). pH-dependent adsorption isotherms of glyphosate, *J.Agric.Food Chem.*, 33 : 1075-1078.
- Meites, L. (ed.). *Handbook of Analytical Chemistry*, McGraw Hill, New York.
- Miles, C.J., Wallace, L.R. and Moye, H.A. (1986). Determination of glyphosate herbicide and (aminomethyl)phosphonic acid in natural waters by liquid chromatography using pre-column fluorogenic labeling with 9-fluorenyl methyl chloroformate, *J.Assoc.Off.Anal.Chem.*, 3 : 458-461.
- Miles, C.J. and Moye, H.A. (1988). Extraction of glyphosate herbicide from soil and clay minerals and determination of residue in soils, *J.Agric.Food Chem.*, 36 : 486-491.

Mitchell,B.,Armstrong,C.,Black,M. and Chapman,J.(1980).
Physiological aspects by sprouting and spoilage in
development of Triticum aestivum L. (wheat) grains, in
Seed Production, Butterworths, London.

Moorby, J.(1968). The influence of carbohydrate and
mineral nutrient supply on the growth of potato tubers,
Ann. Bot., 32 : 57-68.

Moorby, J.(1970). The production,storage and translocation
of carbohydrates in developing potato plants, Ann. Bot.,
34 : 297-308.

Morrison,R.T. and Boyd,R.N.(1968). Organic Chemistry,
Allyn and Bacon, Boston, U.S.A.

Motekaitis,R.J. and Martell,A.E.(1985). Metal chelate
formation by N-phosphonomethyl glycine and related
ligands, J.Coord.Chem., 14 : 139-149.

Moye,H.A. and St.John,P.A.(1980). A critical comparison of
pre-column and post-column fluorogenic labeling for the
HPLC analysis of pesticide residues, in Pesticide
Analytical Methodology (eds.J.Harvey and G.Zweig).
American Chemical Society, Washington. : 89-102.

Moye, H.A., Miles, C.J. and Scherer, S.J. (1983). A simplified high-performance liquid chromatographic residue procedure for the determination of glyphosate herbicide and (aminomethyl)phosphonic acid in fruits and vegetables employing post-column fluorogenic labeling, J.Agric.Food Chem., 31 : 69-72.

Moye, H.A. and Deyrup, C.L. (1984). A simple single-step derivatization method for the gas chromatographic analysis of the herbicide glyphosate and its metabolite, J.Agric.Food Chem., 32 : 192-195.

Nazarova, T.A., Dolinskaya, L.V., Makeev, A.M., Chkanikov, D.I. and Pavlova, N.N. (1988). Determination of glyphosate in plants, Khim.Sel'sk.Khoz., 4 : 42-44. Cited : Chem.Abs., 108-220429.

Nomura, N.S. and Hilton, H.W. (1977). The adsorption and degradation of glyphosate in five Hawaiian sugarcane soils. Weed Res., 12 : 113-121.

Norit (U.K). Introduction to Activated Carbon.

Norris, W.A. (ed) (1953). Crop Dusting : Legal Problem in a New Industry, Stanford Law Rev., 6 : 69-90.

O'Donovan, J.T. and O'Sullivan, P.A. (1982). The antagonistic action of 2,4-D and bromoxynil on glyphosate phytotoxicity to barley (Hordeum vulgare), Weed Sci., 30 : 30-34.

O'Keeffe, M.G. (1980). The control of Agropyron repens and broad leaved weeds pre harvest of wheat and barley with isopropylamine salt of glyphosate. Proceedings of the 1980 British Crop Protection Conference - Weeds, 1 : 55-60.

O'Keeffe, M.G. (1980a). The control of perennial grasses by pre harvest application of glyphosate. Proceedings of the Conference on Grass Weeds in Cereals in the United Kingdom, Association of Applied Biologists, Warwick, U.K. : 137-144.

O'Keeffe, M.G. (1980b). The effect of pre harvest application of the isopropylamine salt of glyphosate on treated crops. Proceedings of the Conference on Grass Weeds in Cereals in the United Kingdom, Association of Applied Biologists, Warwick, U.K.: 145-153.

O'Keeffe, M.G. and Makepeace, R.J. (1985). Efficacy of glyphosate in arable situations, in The Herbicide Glyphosate (eds. E. Grossbard and D. Atkinson), Butterworths, London.

Pavoni, G. (1978). Investigation of glyphosate and its principle metabolite, aminomethyl phosphonic acid, by TLC, Bollettino de chimici dell'Unione Italiana Laboratori Provinciali, 9 : 157-161. Cited : Chem. Abs., 90-1478V.

Perrich, J.R. (ed) (1981). Activated Carbon Adsorption for Waste Water Treatment, CRC Press, Florida.

Perrin, D.D. (1979). Stability Constants of Metal-Ion Complexes. Part B : Organic Ligands, Pergamon Press, Oxford.

Pesticide Analytical Manual (1977). Monsanto Chemical Co. Vol.2. Food and Drug Administration, Washington, D.C. Pest.Reg.Sec.180.364.

Pomeranz, Y. and Robbin, G.S. (1971). Malt sprouts. Their composition and use, Brew.Dig., 46 : 58-64.

Putnam, A.R. (1976). Fate of glyphosate in deciduous fruit trees, Weed Sci., 24 : 425-430.

Ragab, M.T.H. (1978). Thin-layer chromatographic detection of glyphosate herbicide (N-phosphonomethyl glycine) and its aminomethyl phosphonic acid metabolite, Chemosphere, 7 : 143-153.

Ragab, M.T.H., Stiles, D.A. and Yeo, J. (1979). The rapid analysis of glyphosate and its major metabolite by molecular emission cavity analysis, Abstract of the 1979 Meeting of the Weed Science Society of America, : 23.

Ringbom, A. (1963). Complexation in Analytical Chemistry, John Wiley & Sons, New York.

Robert, H.A.(ed.) (1982). Weed Control Handbook : Principles, 7th edition, Blackwell Scientific Publication, Oxford.

Robinson, G.W.(1978). Development of ion exchange technique, in Amino Acid Determination : Methods and Techniques (ed.S.Blackburn), 2nd edition, Marcel Dekker, New York.

Robinson, R.A.(1987). Buffer solutions: operational definitions of pH, in CRC Handbook of Chemistry and Physics (ed.R.C.Weast) 68th edition, CRC Press, Florida.

Rom,R.C.and Talbert,R.E.(1973). Field evaluation of herbicide in fruit and nut crops. Report of the Univ. of Arkansas Agric. Experimental Station, 212 : 1-30.

Romanowski, R.R.(1974). Effect of low level of glyphosate on field grown tomatoes, Proceedings of the North Central Weed Control Conference, 29 : 73.

Roseboom,H. and Berkhoff,C.J.(1982). Determination of the herbicide glyphosate and its major metabolite aminomethyl phosphonic acid by high performance liquid chromatography after fluorescence labelling, Anal.Chim.Acta, 135 : 373-377.

Rueppel,M.L.,Suba,L.A.and Marvel,J.T.(1976).

Derivatization of aminoalkylphosphonic acids for
characterization by gas chromatography mass spectrometry,
Biomed.Mass Spectrom., 3 : 28-31.

Rueppel,M.L.,Brightwell,B.B.,Schaefer,J. and
Marvel,J.T.(1977). Metabolism and degradation of
glyphosate in soil and water, J.Agric.Food Chem., 25 :
517--528.

Schwarzenbach,G. and Flaschka,H.(1969). Complexometric
Titrations, 2nd English edition by H.M.N.H.Irving, Methuen
& Co., London.

Seiber,J.N,McChesney,M.M.,Kon,R. and Leavitt,R.A.(1984).
Analysis of glyphosate residue in kiwi fruit and asparagus
using high-performance liquid chromatography of
derivatized glyphosate as a clean-up step, J.Agric Food
Chem., 32 : 687-681.

Sharp, D.W.A.(ed) (1987). Dictionary of Chemistry, Penguin
Books, Middlesex, U.K.

Shaw,K.A.and Bruzzere,E.(1979). Herbicide screening for
control of Hypericum perforatum. Proceeding of the 7th
Asian Pacific Weed Science Society : 185-187.

Shea,P.J. and Tupy.D.R.(1984). Reversal of cation-induced reduction in glyphosate activity with EDTA, Weed Sci., 32 : 802-806.

Sheppard,B.W.,Pascal,J.A.,Richards,M.C. and Grant, H. (1982). The control of Agropyron repens by the pre harvest application of glyphosate and its effect on grain yield, moisture, germination and harvesting. Proceedings of British Crop Protection Conference - Weeds : 953-960.

Shoval,S. and Yariv,S.(1979). The interaction between Round-up (glyphosate) and montmorillonite. Part 1. Infrared study of the sorption of glyphosate by montmorillonite, Clays and Clay Miner., 27 : 19-28.

Smid,D. and Hiller,L.K.(1981). Phytotoxicity and translocation of glyphosate in the potato (Solanum tuberosum) prior to tuber initiation, Weed Sci., 29 : 218-223.

Smith, O.(1968). Potatoes : Production, Storing, Processsing. AVI Pub.Co.

Sprankle,P.,Meggit,W.F. and Penner,D.(1975a). Adsorption, action and translocation of glyphosate, Weed Sci., 23 : 235-240.

Sprankle, P., Meggit, W.F. and Penner, D. (1975b). Adsorption, mobility and microbial degradation of glyphosate in the soil, *Weed Sci.*, 23 : 229-234.

Sprankle, P., Sandberg, C.L., Meggit, W.F. and Penner, D. (1978). Separation of glyphosate and possible metabolite by thin-layer chromatography, *Weed Sci.*, 26 : 673-674.

Stumm, W. and Morgan, J.J. (1981). *Aquatic Chemistry : An Introduction Emphasizing Chemical Equilibria in Natural Waters*, 2nd edition, John Wiley & Sons, New York.

Snyder, L.R. and Kirkland, J.J. (1979). *Introduction to Modern Liquid Chromatography*, 2nd edition, John Wiley & Sons, New York.

Tollervey, F.E., Paniagua, B.O. and Gonzalez, B.G. (1980). Herbicide trials in annual crops in Santa Cruz, Bolivia 1978-79. CIAT Report, 8 : 28-32.

Tooby, T.E. (1985). Fate and biological consequences of glyphosate in the aquatic environment, in *The Herbicide Glyphosate* (eds. E. Grossbard and D. Atkinson), Butterworths, London.

Topalski, E. and Vulera, R. (1985). Method for identification and determination of glyphosate in biological material, *Vet-Med. Nauki*, 22 : 41-45. Cited : *Chem. Abs.* 103-208198.

Toth,J. and Morrison,G.(1977). Glyphosate drift damages fruit trees, Agricultural Gazette of New South Wales, 88 : 44-45.

Tuinstra,L.G.M. and Kienhuis,P.G.M.(1987). Automated two-dimensional HPLC residue procedure for glyphosate on cereals and vegetables with post-column fluorogenic labelling, Chromatographia, 24 : 696-700.

Twiss,P.T.G. and Jones.M.P.(1965). A survey of wastage in bulk stored maincrop potatoes in Great Britain, Eur.Potato J., 8 : 154-172.

Van Horne,K.C.(ed.) (1985). Sorbent Extraction Technology, Analytichem International, Harbor city, U.S.A.

Vogel, A.I.(1964). A Text-book of Quantitative Inorganic Analysis, 3rd edition, Longmans, London.

Wauchope, D.(1981). Acid dissociation constants of arsenic acid, methylarsonic acid (MAA), dimethylarsinic acid (cacodylic acid) and N-(phosphonomethyl)glycine (glyphosate), J.Agric.Food Chem., 24 : 717-720.

Westerback,S.,Rajan,K.S. and Martell,A.E.(1965). New multidentate ligands. III. Amino acid containing methylenephosphonate groups, J.Am.Chem.Soc., 87 : 2567-2572.

Whistler, R.L. and BeMiller, J.N. (1962). Carbon column chromatography, in Methods in Carbohydrate Chemistry, Vol. 1. (eds. R.L. Whistler and W.L. Wolfrom), Academic Press, New York.

White, R.E. (1981). Introduction to the Principles and Practice of Soil Science, Blackwell Scientific Publications, Oxford.

Wild, A. (1988). Russell's Soil Conditions and Plant Growth, 11th edition, Longmans Scientific & Technical, Harlow, U.K.

Wiese, A.F. (1976). Glyphosate on perennial weeds, Proceedings of the 29th Annual Meeting of the Southern Weed Science Society : 88-93.

Wills, G.O. and McWhorter, C.G. (1985). Effect of inorganic salts on the toxicity and translocation of glyphosate and MSMA in purple nutsedge (Cyperus rotundus), Weed Sci., 33 : 755-761.

Worthing, C.R. (ed.) (1987). Pesticide Manual, 8th edition, The British Crop Protection Council, Thorn Heath U.K.: 449-450.

Worthington,T.R.(1985). The effect of glyphosate on the viability of seed potato tubers, Potato Res., 28 : 109-112.

Wyrill III,J.B. and Burnside,O.C.(1976). Adsorption, translocation and metabolism of 2,4-D and glyphosate in common milkweed and hemp dogbane, Weed Sci., 24 : 557-566.

Yates,W.E.,Akesson,N.B. and Bayer,D.E.(1978). Drift of glyphosate sprays applied with aerial and ground equipment, Weed Sci., 26 : 597-604.

Young,J.C.,Khan,S.U. and Marriage,P.B.(1977). Fluorescence detection and determination of glyphosate via its N-nitroso derivative by thin-layer chromatography, J.Agric.Food Chem., 25 : 918-922.

Zandstra,B.H. and Nishimoto,R.K.(1977). Movement and activity of glyphosate in purple nutsedge, Weed Sci., 25 : 268-274.